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The functional response to fluctuations in water quality of indigenous periphytic algal populations colonizing tubular substrates in lotic waters.

Bruce Edward Tease
University of Massachusetts Amherst

THE FUNCTIONAL RESPONSE TO FLUCTUATIONS IN WATER QUALITY OF INDIGENOUS PERIPHYTIC ALGAL POPULATIONS COLONIZING TUBULAR SUBSTRATES IN LOTIC WATERS

A Thesis Presented

By

BRUCE EDWARD TEASE

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Dr. Robert Walker, Member

Dr. Richard Damon, Member

Dr. Allen V. Barker, Dept. Head
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To my Lord,

for what we endeavor

but credit not

To my parents,

who have been my rock

and Phyl, my refuge

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I wish to extend a heartfelt appreciation to my mentor and confidant, Dr. Robert A. Coler, for his guidance and thoughtful criticisms throughout this research. To the members of my committee, Dr. Robert Walker and Dr. Richard Damon, I give thanks for their assistance during this work. I am especially in debt to Simon Zatyrka for his many helpful hours in the field and in the laboratory, as well as for his full time friendship. Last but not least, I wish to thank those fellow students who have assisted in this research and specifically for their friendship, which I will never forget.

ABSTRACT

THE FUNCTIONAL RESPONSE TO FLUCTUATIONS IN WATER QUALITY OF INDIGENOUS PERIPHYTIC ALGAL POPULATIONS COLONIZING TUBULAR SUBSTRATES IN LOTIC WATERS

(May 1983)

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A procedure for monitoring water quality in lowland brooks and streams is proposed. Alterations in an algal community's capacity to generate O_2 under varied conditions indicated a graduated biological response to water quality. The magnitude and direction of these changes were interpreted as justification for further, more detailed testing. Two streams in Amherst, Massachusetts were selected to determine the practicality, sensitivity and reproducibility of the tubular artificial substrate technique as an index of both cultural eutrophication and toxicity.

The Fort River was selected to study the protocol's sensitivity to a tertiary sewage treatment discharge. Two sets of substrates were deployed in the stream above and below the outfall to be eventually colonized by indigenous

tycoplankton. The 1.8 fold enhancement in O_2 generation by the algal community below the outfall was attributed to nutrient enrichment.

Coal leachate seepage into Taylor Brook served to explore the algal community's response to toxicity. Tubular substrates were colonized by the indigenous periphyton of a clean water tributary of Taylor Brook. One set (3 replicates) of tubes was subsequently transferred to the brook while the other remained in the tributary as the control. Oxygen evolution in the Taylor Brook set precipitously dropped 85% in 5 days and was eliminated after 10 days. Periphyton respiration, though not eliminated, proved to be suppressed at the close of the exposure period. An investigation into the chemistry of Taylor Brook uncovered low pH's (x=4.4) and abnormally high aluminum concentrations (x=3.8ppm) as probable sources of stress.

Substrate productivity was computed from the differential generated between influent and effluent dissolved oxygen concentrations. Oxygen evolution was recorded as mg/l, converted to ug/sec and $mg/m^2/hr$ for comparison between assays and referenced research. Despite the greater quantity of O_2 generated in the larger field substrates (more biomass), smaller diameter substrates with an enhanced surface area to volume ratio, and thus less dilution provided a favorably greater DO differential. Larger dif-

ferentials permitted greater sensitivity in the detection of stressed conditions.

A laboratory version of the protocol lessened the degree of variability between substrates, served to verify field observations and permitted exposure of the substrate community to amended test water. An assay with Taylor Brook water indicated that though the impact of aluminum and pH was dramatic, pH alone was sufficient to eliminate productivity and depress respiration. Long term exposures (1 month), at a constant light intensity of 500 ft-c, revealed daily oscillations in O_2 evolution but of lower amplitude than observed in the field.

The technique's capacity to assay cultural eutrophication, pH and aluminum toxicity offers promise as a sensitive inexpensive tool to routinely evaluate lotic environments, in the field as well as in the laboratory. The option to alter physical conditions and amend test waters in the laboratory, expanded its versatility to assess the biological response to stressed conditions.

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INTRODUCTION

Streams and brooks traverse forests and crop lands across the country, accounting for an important supplement to our larger rivers. Aquatic life, as well as terrestrial wildlife evolve around the reach of the lowland brook. Perhaps of more economic importance, these small tributaries are fundamental to livestock and farmers who rely heavily on the continued supply of potable water. Unfortunately, these small but significant systems are prone to contamination from a variety of "villains". Leachates from dump sites past and present, inevitably make their way to stream banks, depositing an endless array of heavy metals, pesticides and toxic chemicals. Heavy rains transport leachable pollutants from soil to stream, with non point-source runoff introducing similar pollutants. Monitoring these numerous low order tributaries for hazardous contaminants is an insurmountable task. Inaccessibility to many lotic habitats restricts the application of appropriate sampling strategies. Consequently a sampling regime is often established based on convenience rather than necessity. For a continually flowing system receiving pollutants intermittently, a random sampling sequence often provides inaccurate results.

The intention of the author was to develop an appropriate procedure based on variations in relative net primary productivity between two locations (test and control) in monitoring rural lotic systems for stress and nutrient enrichment. Cultural eutrophication and toxicity encompass the range of concerns vital to water pollution control. These problems were explored to determine the practicality and sensitivity of the tubular artificial substrate technique, in the field as well as in the laboratory. This procedure should prove to be most attractive to pollution control agencies because of its sensitivity, mobility and economy.

LITERATURE REVIEW

Lotic monitoring practices generally employ water chemistry and ecological analyses as inventory tools. Appropriate parameters such as phosphorous, nitrogen, inorganic carbon, pH, dissolved oxygen and toxic materials reflect the general abiotic condition of a system. The biotic community (periphyton, macrophytes, macroinvertebrates and fish) is comprised of individuals whose basic life requirements are interfacing with the present abiotic condition (Whitton,1975). Unfortunately, most water chemistry analyses base measurements on isolated sporadic sampling events. For a continually flowing system, receiving an intermittent discharge of pollutants, a periodic sampling regime often generates ambiguous data. An exorbitant effort must be made to thoroughly sample such a system to obtain realistic results. Consequently, stream monitoring practices heavily rely on structural comparisons (composition and distribution) of biotic communities. Diversity indexes are designed to relate the extent and proportion, biotic communities respond to stressed conditions. The response to pollutants does not reflect isolated exposures, but rather the composite of a total exposure over an extended period of time.

The response of lotic algae to stress has become a major focus in stream monitoring practices. The periphytic algal populations of lotic habitats have been exploited as indices of water quality since Kolkwitz and Marsson (1908) resorted to them in their Saprobien System. This is in a large measure due to their unique role as virtually the sole components of the lotic community with the capacity to accumulate nutrients or toxicants. The emphasis in diagnosis has since shifted, however, from structural to functional aspects of algal communities (Cairns et al,1973; Cummins,1974; Odum,1964) with photosynthesis and respiration the most responsive parameters at the ecosystem level (Odum,1977). Estimating a metabolic response to various water quality parameters permits correlating chemical conditions to biological effects.

Primary productivity in lotic systems has been studied through a wide variety of techniques. Direct analyses such as the open stream diurnal method (Odum,1956), the Liebig cooling tubes for periphyton O_2 production on aquatic macrophytes (Assman,1953) and the enclosed plexiglass skylight dome, anchored to the stream bed (Pennak & Lavelle, 1979) base results on the natural system. Analyses such as chlorophyll determination (McConnell,1959; Waters,1961), tracer techniques (Elwood & Nelson,1972), assays of ATP levels (Holm & Hansen,1973) and mathematical modeling

(Kelly,1974) indirectly provide insights into primary productivity.

Unfortunately, communities vary between locations due to a host of uncontrollable environmental prerequisites (i.e. substrate composition & solar radiation). Thus the exploitation of naturally occuring communities as indicators of stress or primary productivity analysis, appears to be of limited practicality. Methods to overcome this limitation include artificial substrates (Hentschel,1916; Patrick, Holn & Wallace,1954; Tippett,1970; Lampkin,1982), laboratory stream tables (McIntire et al,1964; Gerhardt et al,1977), productivity/respiration chambers (Thomas,1966; Pfeifer,1975 Marker,1976) and the algal assay procedure: bottle test (U.S. EPA,1971). Essentially these procedures allow the investigator to measure particular lotic parameters with the advantage of maintaining a degree of control over environmental variables. However, there exists the tendency of failing to mirror the natural habitat that these artificial systems attempt to model (McItire,1975). One must weigh the reproducibility gained from these procedures against the potential loss in applicability to field conditions.

Several reviews of methods and measurements of algal productivity in stream habitats supplement the existing literature with variations on the original works (Vollenweider,1969; Weitzel,1979; Sladeckova,1962). The decision

regarding which procedure to use depends on the specifications of the situation as well as the preference of the researcher.

OBJECTIVE

Primary productivity is important not only in the bioenergetics of the lotic ecosystem but also as a biological indication of the extent of stress imposed on the community. A review of the literature indicates a variety of methodologies have been evolved to measure this unique parameter. Despite the numerous procedures available, most can be categorized into two groups: structural and functional. Structural techniques evaluate the diversity and richness of a community but require great time and expertise in enumeration and identification. Functional protocols provide valuable insight, not only into what type of organisms predominate, but into their metabolic behaviour as well. The apparatus developed, however, for such analyses become highly specialized as well as expensive. Pollution control agencies, very often can not afford the manpower nor the monetary expense required to implement an adequate lotic monitoring program. Accordingly, the objective of this research was to:

1) Develop a simple, inexpensive field method for detecting the response of periphytic algal

communities to impaired water quality.

2) Determine the practicality, sensitivity, versatility and the statistical reliability of the technique by assaying the eutrophication potential of a tertiary sewage treatment facility on the Fort River and the toxicity of acidic coal leachate in Taylor Brook.

3) Investigate the feasibility of incorporating the field technique into a controlled laboratory regime to confirm conclusions generated in the field.

PROCEDURE

4.1 Study Area

Two streams in the Amherst, Massachusetts area were chosen for study sites. Both streams meander through prime Connecticut River Valley farm lands and provide a critical water supply for dairy and agricultural farming.

The Fort River (Fig. 1) head waters originate in the Pelham Hills, as a stoney mountain trickle (42 18' N, 72 30' W). After a 30 km passage through mixed hardwood forests, cultivated fields and grazing lands, it enters the Connecticut River in Hadley, Massachusetts as a fourth order tributary. The study area was located at Amherst Fields, a small housing project isolated amidst grazing land and mixed hardwood forests, 15 km upstream from its confluence with the Connecticut River. The housing project possesses a tertiary sewage treatment facility, whose effluent sporadically enters the Fort River. It was this very pattern of flow that prompted the selection of the study site to test the protocol's response to cultural eutrophication. Any attempt to extrapolate from river data generated by routine grab sampling would have yielded serious inconsistencies. Sampling sites were located 100 m above and below the sewage plant outfall. Both areas were

essentially similar with respect to insolation, flow rate (6 cfs) and temperature. Water quality in the Fort River was generally good with dissolved oxygen consistently above 5 ppm and a circumneutral pH. Conductivity (50-120 umhos/cm) was characteristic of unbuffered waters. Phosphate phosphorous averaged 0.025 ppm, nitrate nitrogen and chloride were less than 0.2 and 10.0 ppm, respectively. Turbidity was normally less than 5 JTU and the summer average water temperature was 20 C (Fischer,1977). During the test period, the total P and N in the sewage outfall averaged 0.31 and 31.67 ppm, respectively (Table 1). The stream bed at the test and control sites consisted of fine grained sand and silt to small gravel areas. Eroding, three foot banks and fallen trees, together with dense terrestrial vegetation characterized the stream channel.

Taylor Brook (Fig. 2) drains the foot hills surrounding Hawley swamp, in North Amherst, Massachusetts (lat. 42 24' 1", long. 72 30' 10"). The brook in size is rather insignificant, with an average flow of less than 1 cfs (test area) and a total length of 5 km, where at its terminus, it joins Amethyst Brook forming the Fort River. During its short journey, the brook passes through agricultural and grazing land, utilized mostly by dairy cattle. The test area was located 2-3 km from its origin. It is in this area where the stream's physical, chemical and biological pro-

perties are greatly altered. The coal to fire the University of Massachusetts' power plant is stock piled on a hill overlooking the test area. Taylor Brook passes 20 m below the coal pile where it receives leachate seepage (Taylor Brook Survey,1982). The test site was located downstream, 1000 m from the coal pile, 50 m west of the point where Taylor Brook crosses Northeast Street. A clean, ground water fed brook enters Taylor Brook in this area on which the control station was situated 30 m above the confluence. The tributary is only .5 km in length, with a flow rate of approximately .3 cfs. The test and control stations were only 30 m apart, thus permitting a logistically manageable sampling regime. The shrubbery and young woody aspens bordering the streams were cleared at both sites to permit comparable insolation.

"The surficial geology of the area was derived during the Pleistocene Epoch from glacial deposits and sediments of glacial Lake Hitchcock. The deposits, which the coal overlies, are mainly glacial, consisting of unstratified sand and gravel. This fairly porous material offers little hinderance to leachate movement, thus reducing the filtering capacity of the soil." (Taylor Brook Survey,1982). Swamp lands are located throughout the test area. Silt and sand together with occasional stretches of gravel make up the stream bed of Taylor Brook as well as its tributary.

"Underlying the sediments is a bedrock of Amherst schist which possesses a low base status, offering little buffering capacity. The loosely consolidated soils of the area are classified as podzols. These are characterized by high accumulations of iron and aluminum and a pH of less than 5 (Kaplan,1981)" (Taylor Brook Survey,1982).

4.2 Rationale

The protocol to evaluate stress incurred by a lotic community was predicated on comparing relative primary productivity rates between periphyton communities colonizing tubular artificial substrates, located in control and inflicted sites of a stream. The "floral memory" of an algal community was exploited for monitoring intermittent stream conditions. The altered capacity to generate oxygen was interpreted as a biological response to the abiotic conditions.

4.3 Apparatus

The tubular artificial substrate is comprised of clear lexan tubing, with 20 cm diameter wire screened (4 mesh) plastic funnels at the upstream ends. Taylor Brook substrates (4.0 cm x 0.9 cm i.d.) were smaller than those used in the Port River (7.5 cm x 1.25 cm i.d.). The assembly (3 replicates) was secured at each end and middle to hori-

zontal wooden supports lashed to stakes driven into the stream bed (Fig. 3). Periphyton colonization was generally accomplished within 3 weeks. An average flow rate of 3 ml/sec resulted from funnel deflection of stream flow through the tubes. The greater pressure head necessary for sample collection and flow rate measurement, however, required raising the tube terminus slightly above the stream. A weir, constructed to provide a 2-4 cm head at the downstream ends of the substrates (Fort River) facilitated these measurements by providing a continual pressure differential. Flow rates were adjusted by varying the head (raising or lowering tube terminus). In Taylor Brook, head was achieved by a 10 liter reservoir at the upstream end that was continually supplied with stream water (during sample collection) by a battery operated pump. A rubber tubing segment attached to the reservoir valve was successively connected during the sampling regime to each of the substrates. A range of flow rates (0-14 ml/sec) could be achieved by adjusting the valve or height of the substrate terminus. The weir assembly provided flow rates ranging from 2-9 ml/sec. The reservoir system, with finer adjustment capabilities, provided a more consistent flow rate of 6 ml/sec. This was determined to be well within the range of delivery rates (4-10 ml/sec), at which primary produc-

tivity remained relatively independent of flow (Fig. 4). Flow rates were obtained with a graduated cylinder and stop watch. The dissolved oxygen (DO) concentrations were determined by the Alsterberg Azide modification of the Winkler method. Rubber tubing was attached to each substrate end upon sampling. The tubing was thrust to the bottom of a BOD bottle and maintained there until twice the volume was displaced. Samples were immediately fixed and titrated in the field. Dissolved oxygen analysis performed on an uncolonized substrate provided an estimate of the influent oxygen concentration and accounted for any inaccuracies incurred by the technique. Measurement of community respiration was performed with a greater diameter black opaque plastic tube, of equal length that was slipped over the clear colonized substrates.

Upon standardization of the field technique, the system was converted into a compact controlled laboratory version. Conclusions gathered in the field were then subjected to verification in the laboratory. The apparatus consisted of vertically hung glass tubing units (.3 cm i.d. x 366 cm) coiled into helices (10 cm x 70 cm) and colonized by stream periphyton. To establish the community in the coils, stream water was released through the coils for two weeks to achieve the desired colonization. A polyethylene 55 liter carboy above the coils functioned as a reservoir

and pressure head, supplying a flow of 10 ml/min. This was determined as the minimum delivery rate at which productivity remained independent of flow (Fig. 5). Sample collection and flow rate measurement were similar to field procedures.

According to McIntire & Phinney (1965), at lower light intensities (500 ft-c), photosynthesis is independent of temperatures between 8-21 C and CO_2 concentrations of 2-45 mg/l, but sufficient to yield significant productivity. Therefore, a uniformly distributed light source of 500 ft-c was provided by two fluorescent lamps. At this intensity, significant alterations in productivity, due to CO_2 and temperature variations would be minimized.

4.4 Field Collections

Assay Water

Control and test waters were collected for water chemistry analyses and laboratory assays. Polyethylene carboys and liter containers were used to transport water back to the laboratory. The carboys were filled by a battery operated pump, while liter sample bottles were simply immersed into the stream by hand. Carboys and sample bottles were previously washed with 5% hydrochloric acid and rinsed with deionized water.

Chemical Analyses

Water chemistry, temperature and light intensity were measured during the various assays to identify possible relationships between control and test community productivity variations. Stream water was collected during wet and dry periods to estimate the range of exposure conditions to the algal communities. Once a general baseline was established, wet chemical analyses were discontinued. Light intensity and water temperature, however, were performed on site during each substrate sampling sequence.

The analytical procedures were derived from Standard Methods for the Examination of Water and Wastewater, 15 th edition (1980). These were: spectrophotometric analyses of; ortho and total phosphorous, determined by the stannous chloride and persulfate digestion technique, nitrate and ammonia nitrogen by cadmium reduction and Nesslerization, respectively. Sulfate and aluminum were determined by the turbidimetric procedure and the Eriochrome Cyanine R method, respectively. Acidity (CO_2), alkalinity, hardness and calcium were determined titrametrically, while pH was tested with a Fischer, Accumet selective ion analyzer, model 750.

Biological

Two sets (3 replicates) of artificial tubular substrates were utilized for each assay. After a 2-3 week period of periphyton colonization, the tubes were monitored for community structure, chlorophyll-a content and algal productivity. Tube sections 2 cm in length were removed from comparable portions of each tube at the start, middle and close of the testing period. Segments, placed in glass vials were set in a dark, ice packed container and quickly transported to the laboratory. Periphyton were dislodged from the sections for identification and enumeration determinations with a fine razor and placed in vials containing 10 ml of distilled water with Lugols' iodine as a preservative. Cell counts were performed by the Lackey drop (microtransect) counting method (APHA,1980). Glass fiber filter plugs were used to abrade periphyton from the tube sections for the spectrophotometric determination of chlorophyll-a in the presence of phaeophytin-a (APHA,1980). Cells were macerated in a tissue grinder containing 2 ml of 90% acetone. The extract (8 ml) was subsequently centrifuged at 10,000 RPM for 5 minutes and then analyzed for chlorophyll-a (mg/cm substrate) in a Bausch & Lomb spectrophotometer 21.

Productivity was determined as the rate oxygen was produced by algae colonizing tubular substrates. Dissolved

oxygen (DO) was monitored routinely on each substrate. The differential between influent and effluent DO concentrations was recorded as substrate productivity: the difference between primary productivity and community respiration.

RESULTS

The protocol was evaluated with regard to its applicability as a monitoring tool for routine diagnoses of stress and nutrient enrichment in lotic waters. The various tests were intended to assess the procedure's capacity to respond consistently to nutrient enrichment and aluminum toxicity. The response of the substrate community to such conditions is described. Following these results, a discussion of the data will revolve on general findings as they pertain to the limits of resolution, reproducibility and sensitivity of the technique.

5.1 Fort River Assay

Fort River substrate primary productivity (O_2 production) above and below the sewage treatment facility outfall during the sampling period (August to September-80), is presented in table 2. The differences between the average influent and effluent dissolved oxygen values were recorded in mg/l and converted to ug/sec and $mg/m^2/hr$ for comparison with associated assays and referenced research.

A 1.8 fold enhancement in O_2 evolution (7.45 vs 4.22 ug O_2/sec) by the downstream substrate community was attributed to elevated nutrient levels. The range of O_2

production in the substrates, 15.5-133 mg $O_2/m^2/hr$ ($\bar{X}$=93) proved comparable to values reported by: Odum (1957) 16.7-1800 mg $O_2/m^2/hr$; Phinney & McIntire (1965) 104 mg $O_2/m^2/hr$ and Bott et al (1978) 25.9-380 mg $O_2/m^2/hr$.

Although the variation in productivity over the test period was highly significant (Table 3), all substrate communities fluctuated in a similar pattern (Fig. 6). An analysis of covariance, water chemistry and a graphical representation of the variation in algal productivity are depicted in tables 3, 1 and figure 6, respectively

5.2 Laboratory Copper Bioassay

Adaptation of the field protocol into a condensed laboratory version was effected in an assay during February-82. Table 4 represents the results of a 22 hour bioassay utilizing vertically hung glass tubing units colonized with a unialgal population. Tubes colonized with Ankistrodesmus falcatus were exposed, in duplicate to 5 copper concentrations, ranging logarithmically from .001 to 10 ppm, and a control. A randomized complete block design, 2 way classification, analysis of variance (Table 5) indicated a significant difference between substrates prior to copper treatment. Substrate replicability was greatly improved over field measurements but was deceptively presented by ANOVA due to the limited sampling

sequence. A 43% reduction in variability was evident in a comparison of the coefficients of variation between laboratory (16.7%, table 5) and field (29.4%, table 10) substrates prior to copper exposure. At the close of the test, there was a statistically significant variation due to treatments and sample times but not between duplicates. After comparing the treatment means with the control (Dunnett's procedure, 1955 two tailed test at the 5% level) before and after exposure, however, the data show that only treatment 3 (Table 4) varied significantly from the control, accounting for the ANOVA treatment source of variation. Productivity for all treatments increased after 8 hours, but a comparison of mean O_2 evolution values before and after copper exposure disclosed a decrease in productivity for treatments 2, 3, 4 and no change in treatment 1 and an increase in the community exposed to 10 ppm.

5.3 Taylor Brook Field Assay

The substrate community response to coal leachate seepage was analyzed during the spring of 1982. Oxygen production and water temperature at designated Taylor Brook and tributary stations are tabulated in table 6 and graphically presented in figure 8. Light intensity within the substrate was measured with a Pasco light meter equipped with a submersible fiber optic probe. At the depth of the

substrates (5 cm) light at the stream surface was attenuated 40% and 60% inside the substrate. Levels, standard deviations and number of analyses performed for specific parameters at test and control stations are presented in table 7.

A 10 liter carboy positioned above the substrate funnels was substituted for the weir to improve flow rate calibration. The increased pressure head was established only during substrate sampling. Normally a flow rate of 3 ml/sec was maintained by funnel deflection of the stream through the substrate. Oxygen production was measured during flow rate augmentation (Fig. 4). A range was established (4-10 ml/sec) where flow rate variations exerted little effect on the DO differential. The flow rate was adjusted to 6 ml/sec prior to each sampling sequence.

Primary productivity (4.5 ug O_2/sec) prior to transfer was dramatically reduced 85% in 5 days and subsequently eliminated after 10 days. A randomized complete block design, 2 way classification, analysis of variance (Table 8) revealed no significant difference between substrates prior to exposure in Taylor Brook. A nested, split-plot analysis of variance after the transfer of one set to the test station, revealed a significant difference between stations but no difference within substrate replicates. Chlorophyll-a content and algal community composition are

delineated in figures 9 & 10, respectively. Tube sections were excised weekly from comparable substrate areas, but not from the same general segment. Because the substrates developed a biomass gradient, decreasing from upstream to downstream end, the data permit comparisons between tubes at any one time but not within time. Test substrate chlorophyll-a content was a tenth of control values, whereas a similar but less pronounced ($\frac{1}{4}$) reduction in cell counts parallelled the drop in O_2 evolution. The most pronounced change was due to the persistence of Anabaena sp., Gomphonema sp. and Tabellaria sp. at roughly the same population densities.

Microscopic examination of test and control algal communities revealed the elimination of diatom and cyanophyte motility at the test station, though cell conformation remained comparable in both communities.

5.4 Taylor Brook Acute Laboratory Assay

Additional laboratory analyses were performed during May-82. Stream water collected from the tributary of Taylor Brook was delivered through six glass coils for periphyton colonization. One set was maintained as a control while the other received Taylor Brook water. During the test period, O_2 evolution was measured 9 times (Table 9). After 40 hrs the control and test community productivity increased 11%

and dropped 12%, respectively. Prior to exposure to Taylor Brook water, the algal substrate O_2 production (.5-1.0 ug/sec) was monitored 3 times over a 1.5 hour period. The reproducibility among each substrate and the replicability between substrates were greatly improved over field measurements as in 5.2 . The variability between coils, however was again deceptively exaggerated due to the low number of samples taken. A nested split-plot analysis of variance (Table 10) indicated no variation among substrates.

5.5 Taylor Brook Assay: Alternating Light/Dark Periods

During July-82, O_2 production was monitored during alternating light/dark periods in algal tube communities situated in Taylor Brook and its tributary (Table 11). This assay facilitated the determination of periphyton respiration and its response to Taylor Brook toxicity. After colonization of two substrates in the tributary, one was transferred to Taylor Brook. Dissolved oxygen was monitored in 1-5 minute intervals during the transition between light/dark phases. A period of at least 20 minutes was required to achieve a maximum measure of respiration. Oxygen evolution and community respiration ranged from 3 to 6 ug/sec and 1 to 2 ug/sec, respectively.

At the close of the test period (8/11, 14 and 16) respiration was masked by effluent dissolved oxygen values

exceeding influent values. On 8/16/82, purging the substrate (regulating a faster flow) prior to occluding light, appeared to correct the situation. The elimination of O_2 production was similar to that of previous assays, while community respiration exhibited no inhibition (Fig. 11).

The extent to which algal respiration contributed to community respiration could not be determined. However, prior to transfer to Taylor Brook, the tube community generated a distinctly autotrophic P/R ratio (3-4).

The algal tube community was dominated by Oscillatoria sp., Navicula sp., Achnanthes sp., Gomphonema sp., Bulbochaete sp. and Anabaena sp.. Percent composition and chlorophyll-a content are presented in tables 12 and 13.

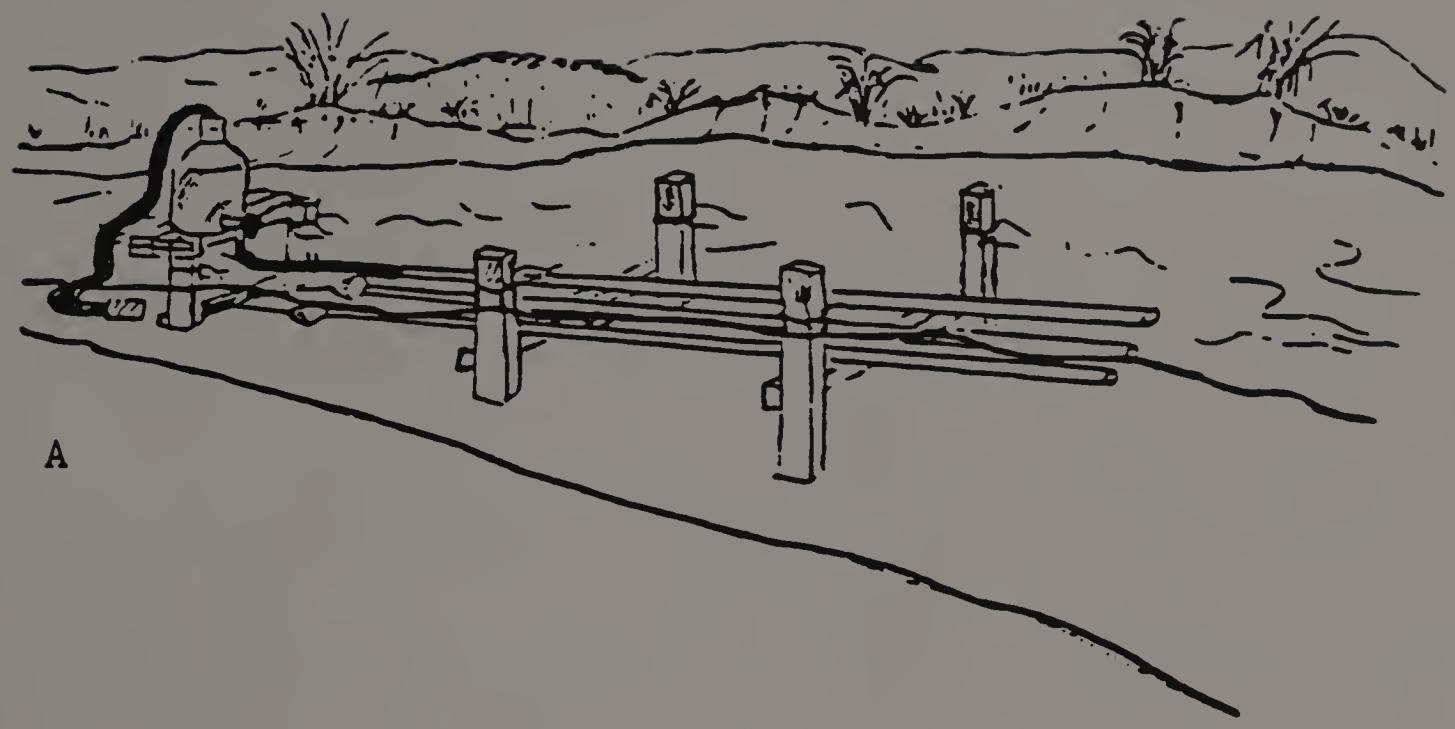
5.6 Taylor Brook Chronic Laboratory Assay

During the month of October-82, the effect of mineral acidity versus aluminum toxicity on periphyton productivity was analyzed in the laboratory. Due to the previously demonstrated low variability in productivity within the glass substrates, the assay was limited to two coils to minimize the required volume of water and to lessen the degree of dissolved oxygen determinations. The communities response to control, test waters and acidified control waters is presented in table 14 and figure 12. The consequence of adding Taylor Brook water to the test coil was

verifiably similar to that observed in the field. Productivity (0.9 ug O_2/sec) precipitously dropped 78% in the first 5 days and more gradually to 94% after the second 5 days. The control coil upon receiving acidified tributary water (4.4 pH) decreased 82% in 5 days. Respiration rates prior to exposure to Taylor Brook water averaged 0.2 ug O_2/sec . After exposure, a 42% decrease in O_2 uptake was noted, whereas a 2 fold increase was observed in the control coil. Similarly, a 38% drop in respiration was detected upon subjecting the control coil to artificially acidified tributary water.

Fig.1 Location map: Fort River Amherst, Massachusetts, depicting test area (*).

Fig. 2 Location map: Taylor Brook Amherst, Massachusetts, depicting control (✱) and test stations (●).



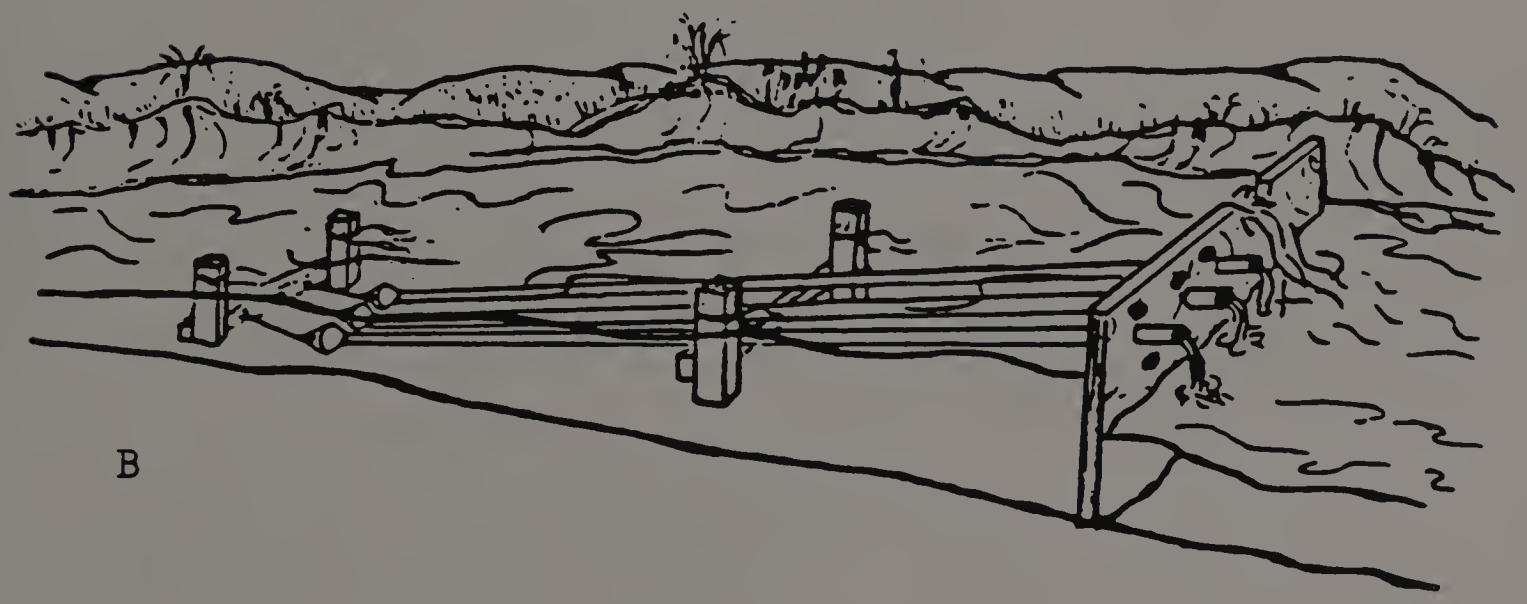


Fig. 3 The tubular artificial substrate assembly, as constructed in Taylor Brook (A) and the Fort River (B).

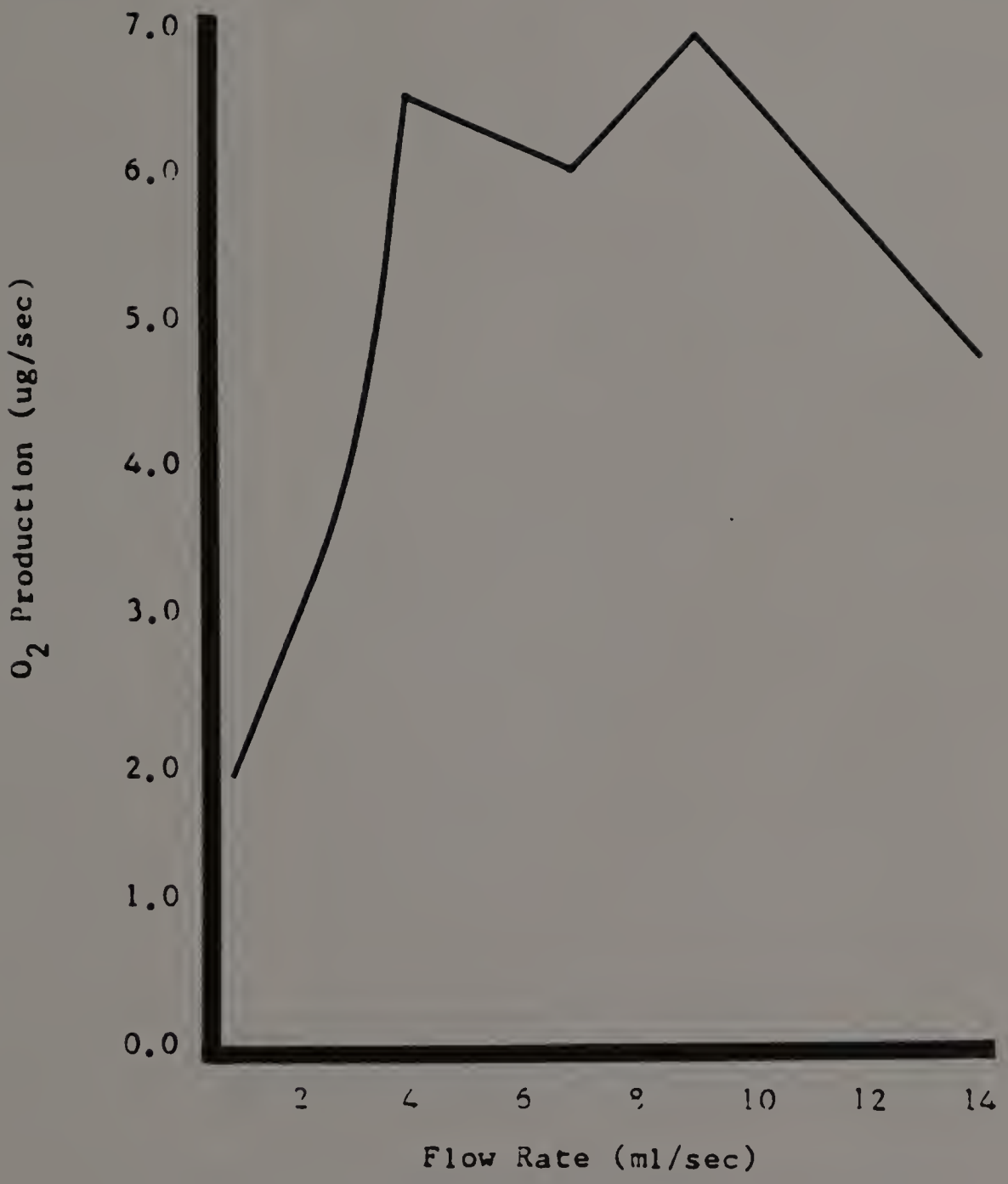


Fig. 4 The response of algal substrate community oxygen production to flow rate variation in the field.

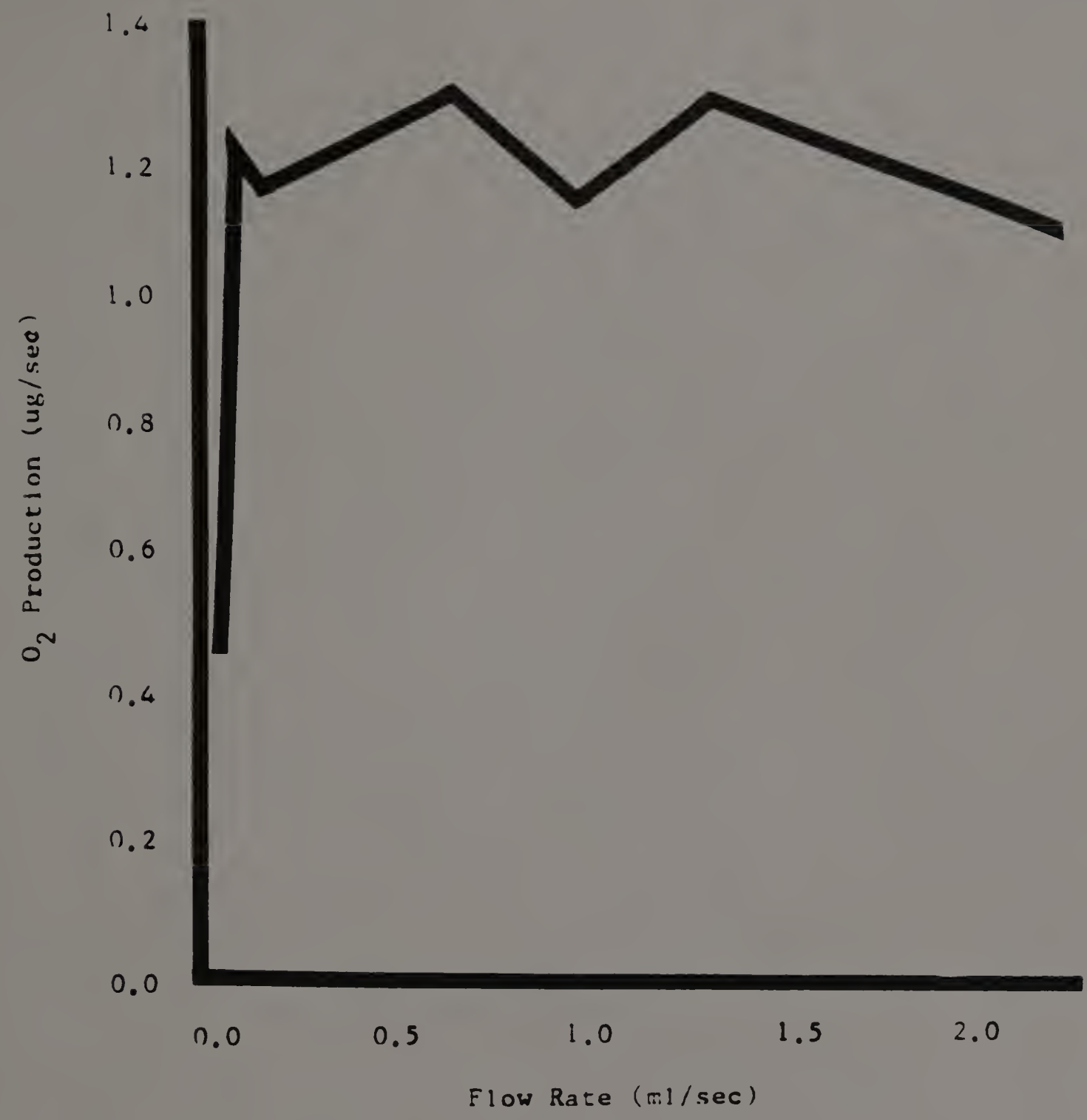


Fig. 5 The response of algal substrate community oxygen production to flow rate variation in the laboratory.

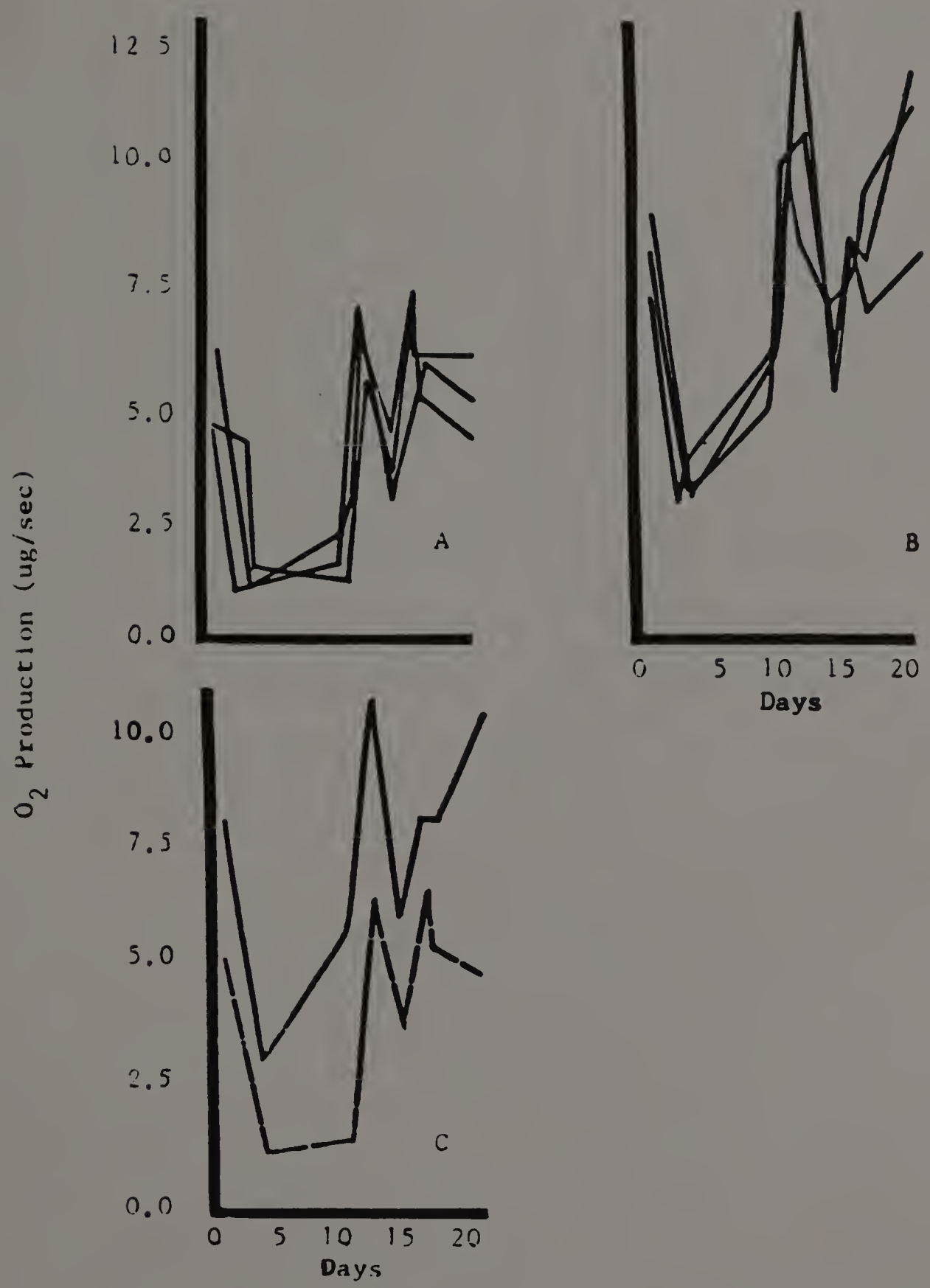


Fig. 6 Rates of replicate O_2 production in the Fort River, above (A) and below (B) the sewage plant outfall. (C: average of A (---) and B (——)).

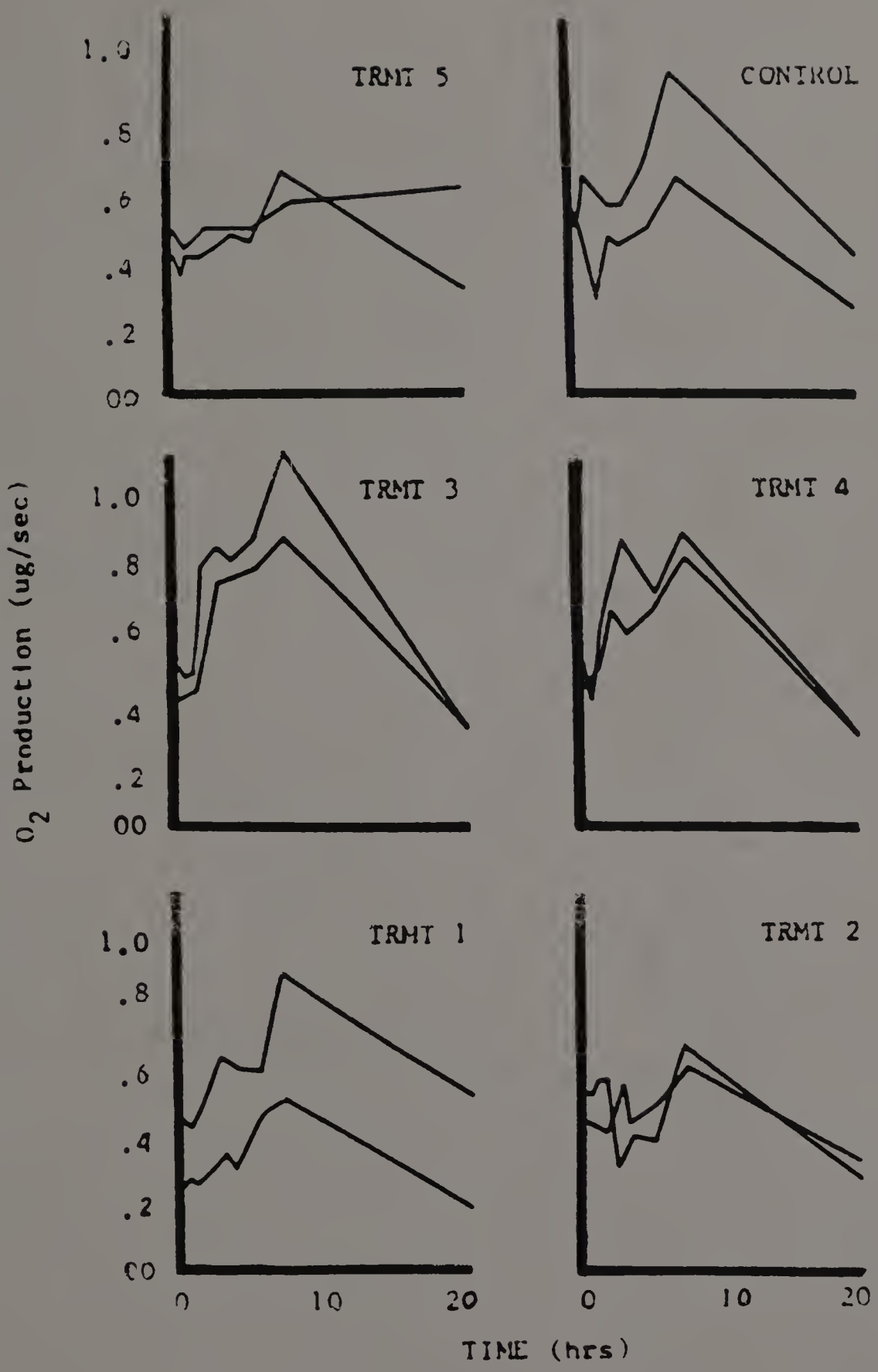


Fig.7 Substrate replicates and treatment responses of O_2 production to copper exposures. Treatments 1-5 represent .001, .01, .1, 1.0 and 10.0 ppm copper.

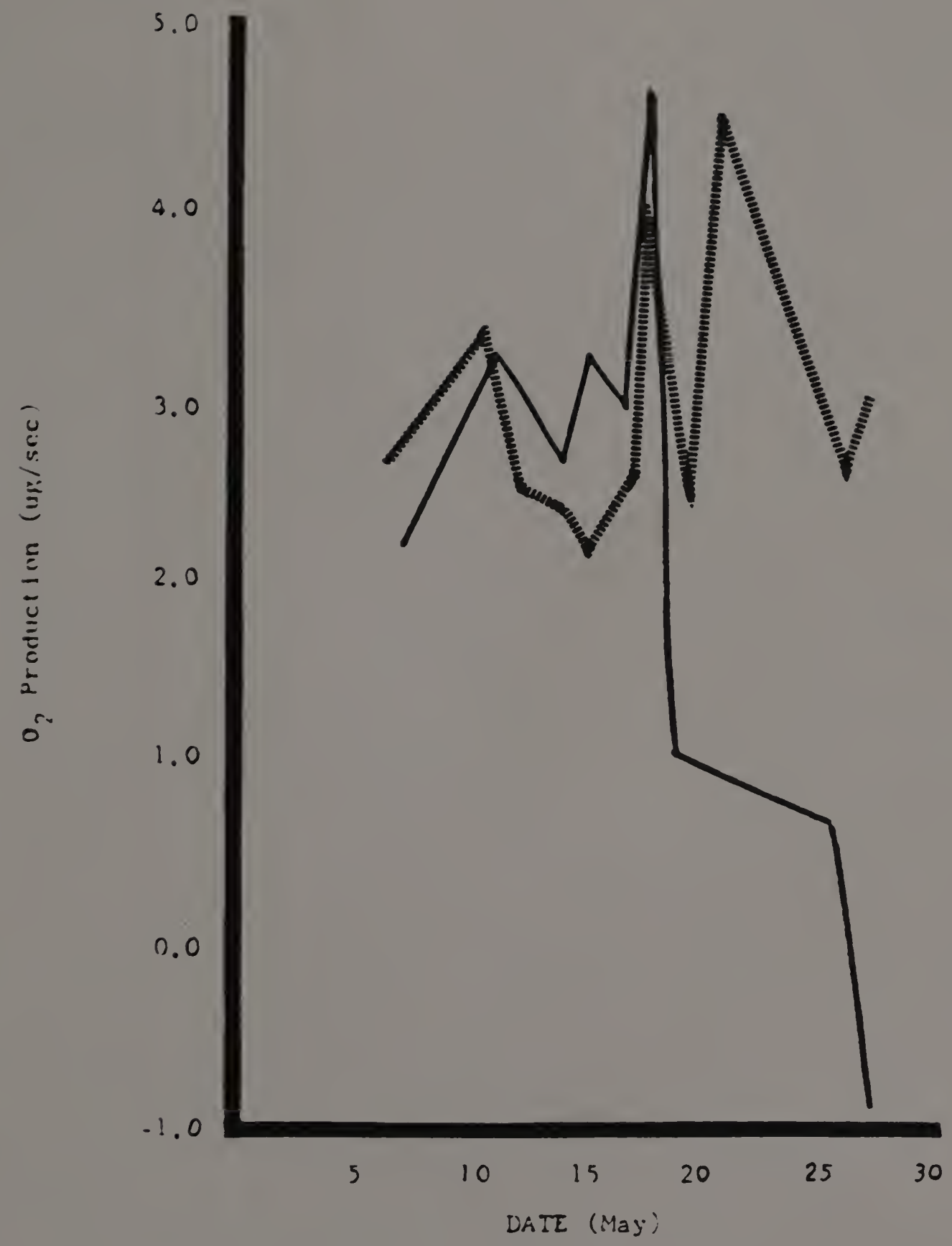


Fig. 8 Rates of average O_2 production observed in substrate communities situated in Taylor Brook (—) and the tributary (▪▪▪▪).

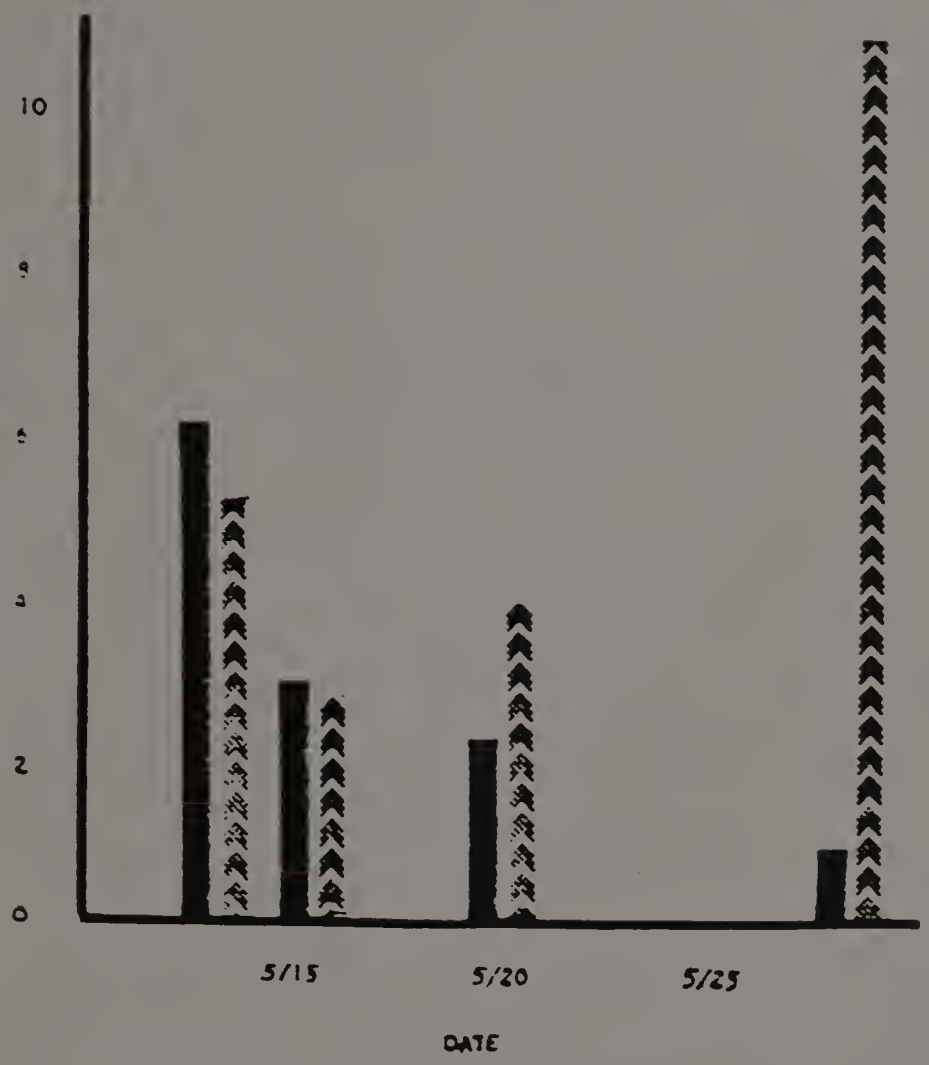


Fig. 9 Average chlorophyll-a concentrations of the Taylor Brook (■) and tributary (▩) algal tube communities.

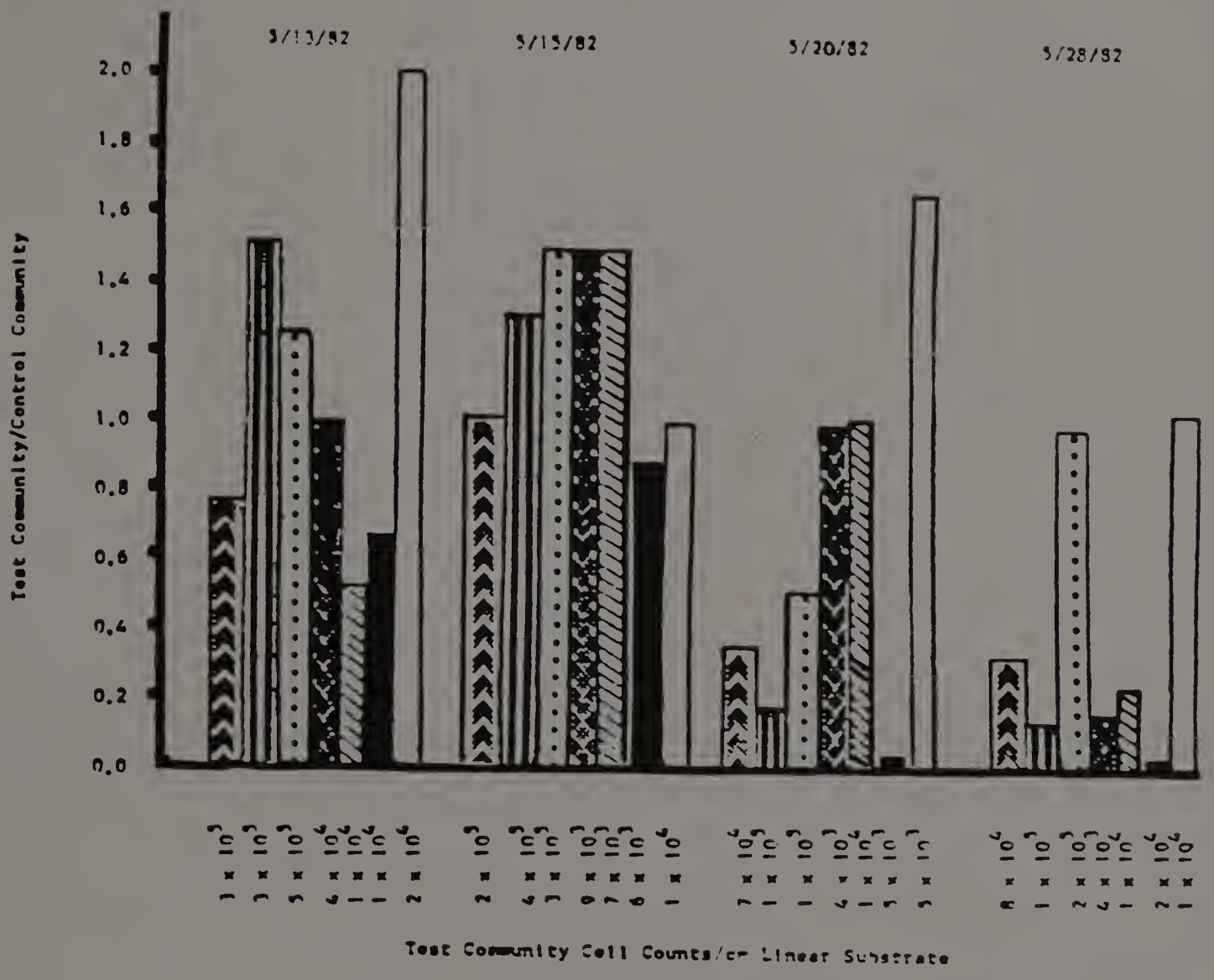


Fig. 10 Substrate periphyton distributions during four sampling periods (average of 3 replicates): Navicula sp. ( ), Achnanthes sp. ( ), Tabellaria sp. ( ), Synedra sp. ( ), Gomphonema sp. ( ), Stigeoclonium sp. ( ) and Anabaena sp. ( ).

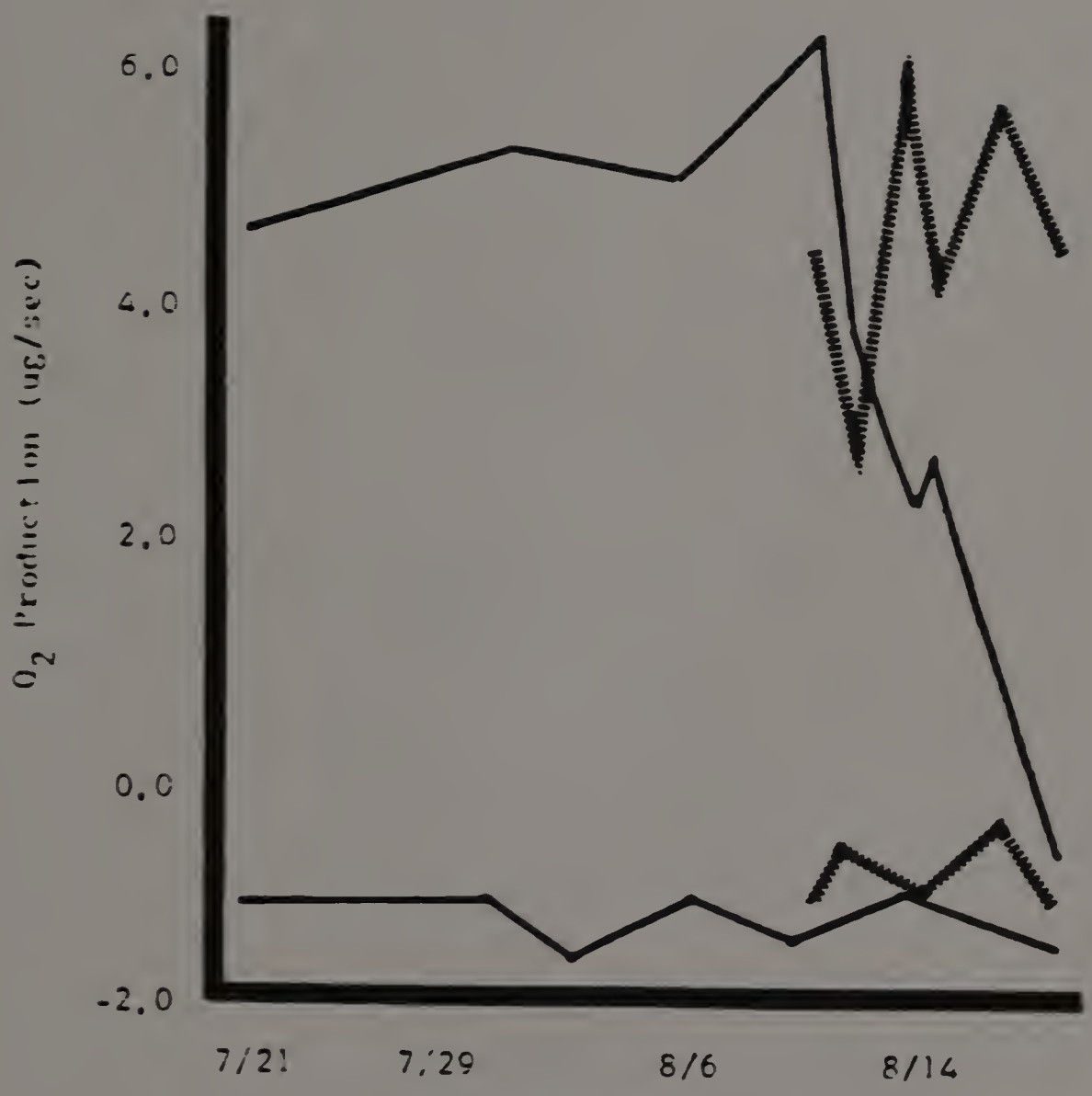


Fig. 11 Average O_2 production during alternating light-dark periods by algal tube communities in Taylor Brook (—) and the tributary (‴‴‴).

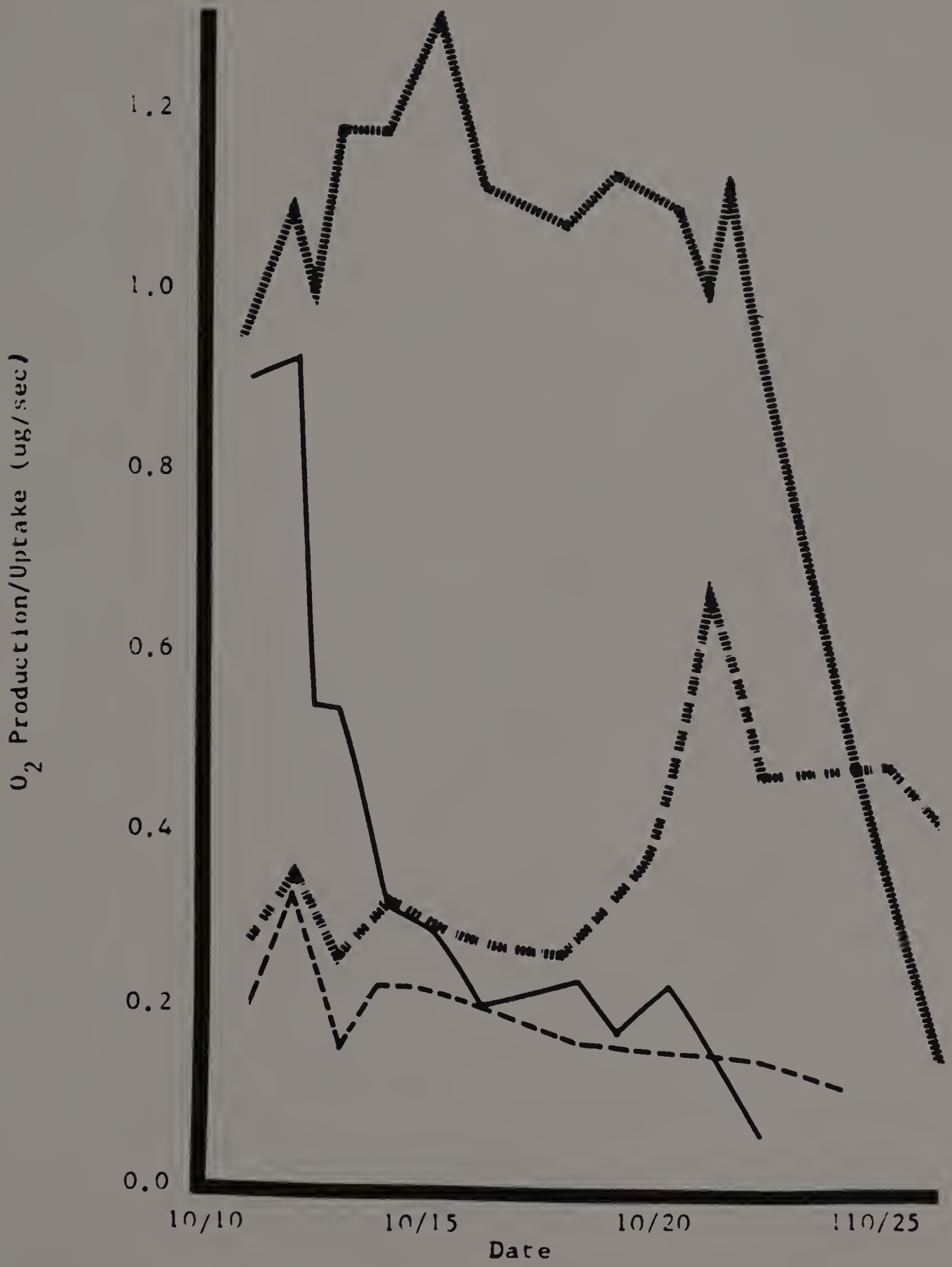


Fig. 12 Oxygen production and uptake by the algal coil communities exposed to tributary (▪▪▪▪▪), Taylor Brook (——) and acidified tributary waters.

VELOCITY PROFILE (cm/sec)

Radius (cm)		Substrate 1	Substrate 2	Substrate 3	$\overline{Av}$
A	0	8.76 (v_{max})	8.90 (v_{max})	8.12 (v_{max})	8.60
B	.1	8.36	8.46	7.72	8.18
C	.2	7.36	7.46	6.72	7.18
D	.3	6.86	6.96	6.21	6.78
E	.4	5.86	5.97	5.25	5.69
F	.45	5.37	5.47	4.75	5.20

$$\text{Flow Rate} = \frac{\pi^2 (R^2 - r^2)}{2\eta}$$

$$v^2 = \frac{v_{max} (2\eta A)}{R^2 - r^2}$$

A = cross sectional tube area (cm^2)

r^2 = distance from center line (cm)

R^2 = tube radius (cm)

η = kinematic viscosity at 37 °C (0.0136 cm^2/sec)

v_{max} = maximum velocity at centerline (measured after 5cm)

v^2 = characteristic velocity dependent on material properties

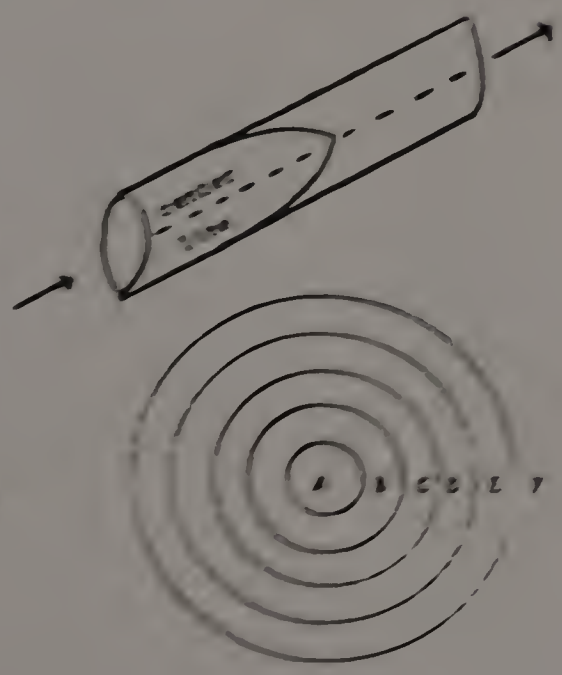


Fig. 13 Flow rate profile for laminar flow through a pipe.

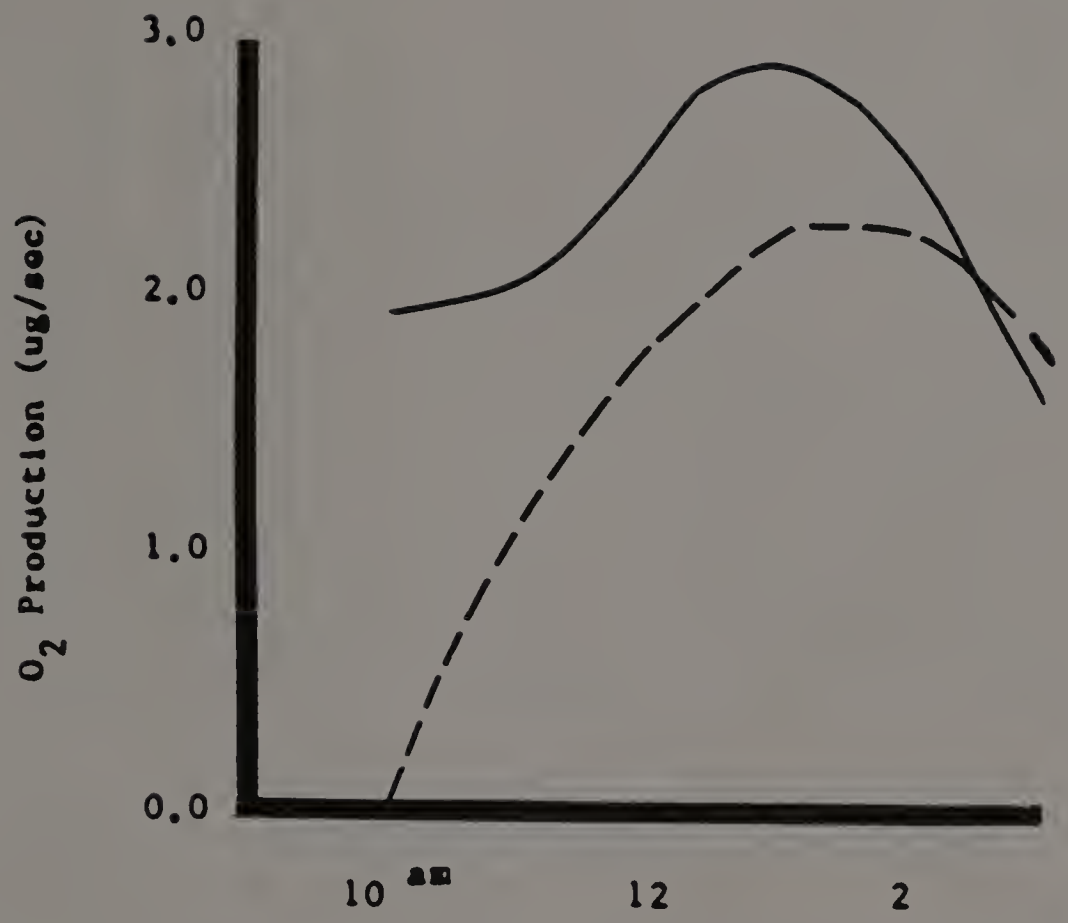


Fig. 14 Average diurnal variation in O_2 production of test (—) and control (--) substrate sets, located in the tributary of Taylor Brook.

PARAMETER	STATION		Sewage Outfall
	Downstream	Upstream	
Total Phosphorous	0.030 (.02)	0.025 (.01)	0.31 (.17)
Nitrate Nitrogen	0.18 (.04)	0.08 (.03)	31.67 (7.43)
Ammonia Nitrogen	0.20 (.15)	0.20 (.10)	0.28 (.28)
BOD (5 day)	0.60 (.20)	0.60 (.20)	1.73 (.97)
Conductivity (umhos)	92	92	- -
Suspended Solids	-	-	1.77 (.55)

Table 1 Levels (ppm) and standard deviations of selected water quality parameters recorded at the sewage outfall (Amherst Fields sewage plant record, summer 1981) and experimental stations (average of 4 analyses).

DATE	STATION										WEATHER
	UPSTREAM					DOWNSTREAM					
	1	2	3	$\bar{X}'$	$\bar{X}''$	1	2	3	$\bar{X}'$	$\bar{X}''$	
8/26	1.3	1.5	2.2			2.3	1.6	1.5			
	4.8	3.1	2.1			3.2	5.6	5.6			
	6.2	4.7	4.6	5.2	64.7	7.4	9.0	8.4	8.3	103.0	sun
8/28	0.7	0.3	1.1			1.0	1.0	1.1			
	3.5	3.3	3.9			2.8	4.8	4.6			
	2.5	1.0	4.3	2.6	32.2	2.8	4.8	5.1	4.2	52.8	cloud
8/29	0.4	0.3	0.3			1.3	0.5	0.7			
	3.1	3.5	4.8			2.9	5.9	4.6			
	1.2	1.1	1.4	1.2	15.5	3.8	3.0	3.2	3.3	41.4	rain
9/5	0.3	0.3	0.2			1.4	0.8	0.9			
	7.7	4.0	6.7			4.5	7.7	5.6			
	2.3	1.2	1.3	1.6	20.2	6.3	6.2	5.0	5.8	72.9	rain
9/6	0.4	0.8	0.6			1.6	1.2	1.5			
	8.3	4.4	9.1			5.3	8.3	6.7			
	3.3	3.5	5.5	4.1	51.3	8.5	10.1	10.1	9.5	118.7	sun
9/7	1.1	1.3	1.1			3.0	2.1	2.2			
	5.9	4.6	6.3			4.4	3.9	4.8			
	6.5	6.0	6.9	6.5	80.8	13.2	8.2	10.6	10.7	133.0	sun
9/9	1.0	1.0	1.1			1.9	1.8	1.1			
	4.6	3.2	3.3			3.0	3.9	4.6			
	4.6	3.2	3.6	3.8	47.6	5.7	7.0	5.1	5.9	74.1	sun/ cold
9/11	1.3	1.9	1.8			2.8	2.2	2.4			
	5.3	2.7	4.2			3.0	3.5	3.5			
	6.9	5.1	7.6	6.5	81.6	8.4	7.7	8.4	8.2	108.1	sun
9/12	1.3	1.1	1.1			3.1	3.3	1.9			
	4.6	5.3	4.6			2.6	2.9	3.6			
	6.0	5.9	5.1	5.6	70.3	8.1	9.6	6.8	8.2	102.0	sun/ warm
9/16	0.9	0.8	0.9			2.5	1.9	1.2			
	6.7	6.3	4.8			4.8	5.9	6.7			
	6.0	5.0	4.3	5.1	64.1	12.0	11.2	8.0	10.4	130.2	sun
		$\bar{X}$ =		4.2	52.8				7.5	93.0	
		s =		1.9					2.5		
		CV =		45%					34%		

Table 2 Oxygen production in the Fort River tubular substrates, with corresponding flow rates (ml/sec). Recorded as mg/l, converted to ug/sec (X') and $mg/m^2/hr$ (X").

Analysis of Covariance

Sources	d.f.	SS	MS	F
1. Station	1	8.6742	8.6742	21.71 ++
2. Tubes:Station	4	1.1037	0.2760	4.31 ++
3. Days	9	14.2004	1.5778	24.66 ++
4. Station x Days	9	1.1660	0.1296	2.02 ns
5. Regression	1	0.6575	0.6575	10.27 ++
6. Error	35	2.2400	0.0640	

Table 3 Analysis of covariance of oxygen production (mg/l) and flow rate (ml/sec) for substrates positioned in the Port River.

TIME (hrs)	TREATMENT (Copper concentration ppm)												
	Control (0.0)		1 (0.001)		2 (0.01)		3 (0.10)		4 (1.0)		5 (10.0)		
	X	X'	X	X'	X	X'	X	X'	X	X'	X	X'	
0	3.2	.53	1.6	.27	3.2	.53	3.1	.51	2.7	.45	2.5	.42	
	3.2	.53	2.8	.47	2.7	.45	2.5	.42	3.1	.51	3.1	.51	
.5	3.2	.53	1.8	.30	3.1	.51	2.8	.47	2.9	.48	2.2	.37	
	3.3	.54	2.7	.45	2.7	.45	2.6	.43	2.7	.45	2.9	.48	
1	4.0	.66	1.7	.28	3.4	.57	2.9	.48	2.8	.47	2.5	.42	
	3.2	.53	2.9	.48	2.7	.45	2.6	.43	2.4	.40	2.6	.43	
2	3.8	.62	2.0	.33	3.5	.58	4.8	.80	3.3	.55	2.5	.42	
	2.1	.34	3.4	.57	2.5	.42	3.6	.60	3.8	.63	3.0	.50	
3	3.5	.58	2.2	.37	2.1	.35	5.3	.88	4.1	.68	2.7	.45	
	2.9	.48	4.0	.67	3.4	.57	4.6	.77	4.6	.77	3.0	.50	
4	3.5	.58	2.0	.33	2.5	.42	5.0	.83	3.7	.62	2.9	.48	
	2.8	.46	3.8	.63	2.7	.45	4.7	.78	5.4	.90	3.0	.50	
6	4.5	.74	3.0	.50	2.4	.40	5.4	.90	4.1	.68	2.7	.45	
	3.1	.51	3.7	.62	3.1	.52	4.8	.80	4.4	.73	3.0	.50	
8	5.9	.97	3.2	.53	4.2	.70	6.9	1.15	5.6	.93	4.0	.67	
	4.0	.66	5.5	.92	3.9	.65	5.4	.90	5.2	.87	3.4	.57	
22	2.6	.43	1.2	.20	1.7	.28	2.0	.33	1.9	.32	1.9	.32	
	1.6	.26	3.3	.55	2.0	.33	2.0	.33	1.9	.32	3.7	.62	
Treatment Means													
	3.4	.55	2.3	.38	3.0	.49	2.8	.46	2.8	.46	2.6	.44	Before Exposure
	2.1	.35	2.3	.38	1.9	.31	2.0	.33	1.9	.32	2.8	.47	After Exposure

Table 4 Oxygen production before (0-1 hr) and after (2-22 hr) copper treatment, in duplicate. Recorded as mg/l (X) and converted to ug/sec (X').

Analysis of Variance

SOURCES	d.f.	SS	MS	F
BEFORE EXPOSURE				
Coil	11	.1820	.0165	14.35 ++
Time	2	.0011	.0006	no test
Error	22	.0254	.0012	
Coefficient of Variation = 16.7%				
AFTER EXPOSURE				
Treatment (Tr)	5	.7341	.1468	15.0 ++
Time (T)	5	1.2582	.2516	22.3 ++
TrT	25	.2458	.0098	.87 ns
Error	36	.4082	.0113	

Dunnett's Procedure

		Pi				
	Control	1	2	3	4	5
TRMT MEAN	.55	.52	.47	.76	.67	.50
CONTROL - Pi	-	.03	.08	.21	.12	.05

$d' = t \sqrt{2(\text{error MS}/\#\text{ of observations per mean}}$

$d' = 2.7\sqrt{2(.0113)/12}$

$d' = .12$

Table 5 Statistical analyses of O_2 generation by Ankistrodesmus falcatus exposed to 5 copper concentrations and a control.

DATE	STATION												AVERAGE O_2 EVOLVED				TEMP (C)
	Taylor						Tributary						Taylor		Trib.		
	1		2		3		1		2		3						
	x	x'	x	x'	x	x'	x	x'	x	x'	x	x'	$\bar{x}'$	$\bar{x}''$	$\bar{x}'$	$\bar{x}''$	
5/7	.36	2.2	.18	1.1	.54	3.2	.54	3.2	.36	2.2	-	-	2.2	57.2	2.7	71.5	15
5/11	.59	3.5	.54	3.2	.51	3.0	.51	3.0	.51	3.0	.65	3.9	3.2	85.8	3.3	87.8	14
5/12	.61	3.7	.15	.87	.72	4.3	.25	1.5	.36	2.2	.61	3.7	3.0	78.3	2.5	64.9	14
5/14	.40	2.4	.59	3.5	.34	2.1	.51	3.0	.36	2.2	.32	1.9	2.7	69.5	2.4	63.1	17
5/15	.50	3.0	.59	3.5	.51	3.1	.51	3.0	.34	2.1	.25	1.5	3.2	84.4	2.2	58.7	17
5/17	.51	3.0	.51	3.0	.36	2.2	.36	2.2	.40	2.4	.55	3.3	2.8	74.3	2.6	70.2	15
5/17	.90	5.4	.74	4.4	.64	3.9	.81	4.9	.60	3.6	.57	3.4	4.6	121.0	4.0	104.9	20
5/18	.18	1.1	.31	1.9	.28	1.7	.39	2.3	.54	3.2	.41	2.4	1.6	41.0	2.6	70.3	14
5/19	-.09	-.55	.05	.28	.28	1.7	.51	3.0	.44	2.7	.25	1.5	.48	12.2	2.4	64.4	17
5/21	-.06	-.34	.18	1.1	.35	2.1	.79	4.7	.72	4.3	.75	4.5	.95	30.8	4.5	119.6	17
5/26	.00	.00	.19	1.2	.17	1.0	.38	2.3	.40	2.4	.51	3.0	.73	19.2	2.6	68.3	17
5/27	-.11	-.68	-.19	-1.1	-.19	-1.1	.60	3.6	.34	2.1	.53	3.2	-.96	-25.9	3.0	78.3	16

Table 6 Oxygen production before and after transfer to Taylor Brook. Recorded as mg/l (x) and converted to ug/sec (x') and mg/m^2/hr ($\bar{x}''$).

PARAMATER	STATION					
	TEST			CONTROL		
	conc.	s	n	conc.	s	n
Phosphorous ortho	0.028	0.021	6	0.034	0.010	8
total	0.058	0.043	8	0.070	0.033	9
Nitrogen nitrate	0.160	0.018	4	0.130	0.036	3
ammonia	0.295	0.116	4	0.180	0.110	5
Suspended solids	5.75	--	1	8.75	--	1
pH	4.49	0.285	7	6.35	0.920	8
Acidity (CO_2)	26.7	16.9	7	22.0	19.7	7
Alkalinity	47.0	22.8	7	76.4	36.1	7
Hardness	33.9	4.18	7	21.0	2.89	7
Calcium	28.2	7.95	5	19.0	1.16	4
Sulfate	42.1	13.9	9	13,6	3.78	9
Aluminum	3.81	0.888	3	0.024	0.029	4

Table 7 Levels (ppm), standard deviation (s) and number of analyses (n) performed for parameters recorded at Taylor Brook test station and the tributary control station (May 1982).

Analysis of Variance

SOURCES	d.f.	SS	MS	F
BEFORE EXPOSURE				
Time	5	2.6212	.5242	no test
Tubes	5	1.9639	.3928	.55 ns
Error	24	17.2722	.7197	
Coefficient of Variation = 29.4%				
AFTER EXPOSURE				
Time (T)	5	40.9795	8.1959	no test
Tubes:Station	4	2.4539	.6135	1.17 ns
Station (S)	1	34.5840	34.5940	8.39 ++
TS	5	20.6185	4.1237	no test
Error	20	10.4687	.5234	

Table 8 Analysis of variance of O_2 production by algal tube communities in the tributary and after transfer to Taylor Brook.

TIME (hr)	STATION												AVERAGE O_2 EVOLVED			
	TAYLOR						TRIBUTARY						TAYLOR		TRIB.	
	1		2		3		1		2		3					
	x	x'	x	x'	x	x'	x	x'	x	x'	x	x'	x'	x"	x'	x"
0.0	3.3	.55	2.8	.46	4.7	.79	3.0	.49	3.9	.65	3.0	.49	.60	62.7	.54	56.9
0.5	3.6	.60	2.8	.46	3.8	.63	3.4	.57	4.0	.66	3.1	.51	.56	59.0	.58	60.6
1.5	3.6	.60	3.1	.52	4.3	.72	3.9	.65	4.4	.74	3.4	.57	.61	64.3	.63	68.1
2.0	5.2	.86	3.0	.49	3.1	.51	3.8	.63	4.6	.77	3.6	.60	.62	64.9	.67	69.7
3.0	3.5	.59	3.0	.49	3.2	.54	4.3	.71	4.9	.82	4.3	.71	.54	56.3	.75	77.8
4.0	3.4	.57	3.1	.51	2.7	.45	4.2	.70	4.4	.74	4.3	.71	.51	53.1	.74	78.3
33.5	3.1	.51	2.8	.46	3.1	.51	4.3	.71	4.0	.66	4.1	.68	.49	52.0	.68	71.9
35.5	3.0	.49	3.0	.49	3.3	.55	4.4	.74	4.3	.71	3.6	.60	.51	53.6	.68	71.3
40.0	3.4	.57	2.8	.46	3.2	.54	3.9	.65	4.1	.68	3.9	.65	.52	54.7	.66	68.7

Table 9 Oxygen production before and after exposure to water collected from Taylor Brook (May 1982). Recorded as mg/l (x) and converted to ug/sec (x') and $mg/m^2/hr$ ($\bar{x}$").

Analysis of Variance

SOURCES	d.f.	SS	MS	F
BEFORE EXPOSURE				
Time	2	.0152	.0076	no test
Coils	5	.1226	.0245	10.65++
Error	10	.0229	.0023	
Coefficient of Variation = 16.9%				
AFTER EXPOSURE				
Time (T)	5	.0200	.0040	no test
Coils:Station	4	.0577	.0144	3.13 ns
Station (S)	1	.2434	.2434	36.88 ++
TS	5	.0328	.0066	no test
Error	20	.0914	.0046	

Table 10 Analysis of variance of O_2 production by algal coil communities in tributary water and after exposure to Taylor Brook water (May 1982).

DATE	O_2 Production					
	Taylor Brook		Tributary			
	time	X	X'	time	X	X'
7/22	0	.73	4.41	-	-	-
	2	.73	4.41	-	-	-
	6	-.08	-.51	-	-	-
	10	.08	.51	-	-	-
	14	-.16	-.97	-	-	-
	20	-.16	-.97	-	-	-
	25	-.20	-1.23	-	-	-
	29	.41	2.45	-	-	-
	33	.66	3.94	-	-	-
	39	.54	3.25	-	-	-
8/2	0	.67	4.19	-	-	-
	2	1.02	6.14	-	-	-
	4	.00	.00	-	-	-
	7	-.04	-.25	-	-	-
	11	-.08	-.51	-	-	-
	21	-.29	-1.73	-	-	-
	31	-.20	-1.23	-	-	-
	42	-.20	-1.23	-	-	-
	43	04	.25	-	-	-
	45	.49	2.96	-	-	-
	52	.94	5.63	-	-	-
	59	1.52	9.10	-	-	-

DATE	O_2 Production					
	Taylor Brook		Tributary			
	time	X	X'	time	X	X'
7/30	0	.86	5.16	-	-	-
	2	.86	5.16	-	-	-
	5	.20	1.23	-	-	-
	10	.00	.00	-	-	-
	16	-.04	-.25	-	-	-
	20	-.20	-1.23	-	-	-
	22	.57	3.43	-	-	-
	24	.82	4.91	-	-	-
	29	.90	5.42	-	-	-
	34	1.02	6.14	-	-	-
	41	1.35	8.09	-	-	-
	44	1.23	7.37	-	-	-
8/6	0	1.07	6.39	-	-	-
	2	.82	4.91	-	-	-
	4	.82	4.91	-	-	-
	6	.04	.25	-	-	-
	10	-.08	-.51	-	-	-
	16	-.20	-1.23	-	-	-
	21	-.25	-1.48	-	-	-
	28	-.20	-1.23	-	-	-
	35	-.20	-1.23	-	-	-
	41	-.20	-1.23	-	-	-
	47	-.37	-2.20	-	-	-
	59	-.20	-1.23	-	-	-
	61	-.12	-.72	-	-	-
	66	.61	3.68	-	-	-
	73	.66	3.94	-	-	-
	79	.98	5.89	-	-	-
	85	.90	5.42	-	-	-
	92	.86	5.16	-	-	-
	99	.78	4.66	-	-	-
	109	.98	5.89	-	-	-

Table 11 Oxygen production during alternating light-dark periods (minutes) by algal tube communities in Taylor Brook and the tributary. Recorded as mg/l (X) and converted to ug/sec (X').

DATE	O_2 Production Taylor Brook time	X	X'	Tributary time	X	X'	DATE	O_2 Production Taylor Brook time	X	X'	Tributary time	X	X'
8/10	0	.94	5.63	0	.73	4.41	8/11	0	.66	3.94	0	.45	2.71
	2	.98	5.89	2	.73	4.41		2	.66	3.94	2	.45	2.71
	4	1.11	6.64					3	.66	3.94	3	.45	2.71
	6	.12	.72	4	.12	.72		6	.04	.25	5	-.12	-.72
	10	.00	.00	13	-.20	-1.23		9	.08	.51	10	-.12	-.72
	17	-.25	-1.48	17	-.12	-.72		14	.16	.97	14	-.08	-.51
	23	-.16	-.97										
	25	.41	2.48	19	.70	4.19		16	.37	2.20	17	.61	3.68
	35	.86	5.16	22	.98	5.89		21	.49	2.96	22	.61	3.68
	40	.90	5.42	27	.98	5.89		26	.66	3.94	27	.98	5.89
	44	.98	5.89	32	1.11	6.64		31	.53	3.18			
8/13	0	.25	1.48	0	.98	5.89	8/14	0	.41	2.46	0	.66	3.94
	2	.32	1.95	2	1.02	6.14		2	.41	2.46	1	.66	3.94
	3	.45	2.71					4	.41	2.46	4	.82	4.91
	7	.20	1.23	6	.20	1.23		8	.41	2.46	6	.00	.00
	12	.20	1.23	10	.04	.25		13	.20	1.23	11	-.12	-.72
	17	-.20	-1.23	15	.00	.00		18	.32	1.95	20	-.20	-1.23
	19	-.20	-1.23	17	.53	3.18		25	.20	1.23			
	25	.08	.51	25	.82	4.91		31	.12	.72			
	30	.12	.72	30	.78	4.66							
8/16	0	.25	1.48	0	.82	4.91	8/18	0	.00	.00	0	.73	4.41
	2	.12	.72	2	1.02	6.14		2	-.12	-.72	2	.61	3.65
	3	.04	.25	3	.94	5.63		3	-.25	-1.48	3	.82	4.91
	5	.04	.25	5	.08	.51		9	-.08	-.51	9	-.04	-.25
	10	.00	.00	10	.20	1.23		15	.25	1.48	15	-.12	-.72
	15	.08	.51	17	-.08	-.51		20	.04	.25	20	-.20	-1.23
	18	.00	.00	21	.66	3.94		23	-.25	-1.48	25	.49	2.96
	25	.12	.72	26	.82	4.91		28	.16	.97	30	.70	4.19
	30	.08	.51	29	.82	4.91		38	-.04	-.25	35	.53	3.18

Table 11 Oxygen production during alternating light-dark periods (minutes) by algal tube communities in Taylor Brook and the tributary. Recorded as mg/l (X) and converted to ug/sec (X').

GENUS	PERCENT COMPOSITION DATE							
	8/10		8/14		8/16		8/19	
Oscillatoria sp.	44'	18"	59	21	10	12	24	22
Navicula sp.	36	33	18	42	35	37	35	45
Achnanthes sp.	8	38	3	21	46	41	23	16
Tabellaria sp.	3	0	1	2	1	0	4	0
Gomphonema sp.	3	5	15	1	2	4	3	10
Fragelaria sp.	2	2	0	4	1	4	1	7
Bulbochaete sp.	2	2	4	2	2	1	1	0
Anabaena sp.	1	2	0	3	3	0	6	0

Table 12 Percent composition of algal tube community dominants in Taylor Brook (') and tributary (") substrates.

DATE	STATION	
	TAYLOR BROOK	TRIBUTARY
8/10	31.9	35.3
8/14	28.8	42.7
8/16	13.6	36.0
8/19	21.8	34.2

Table 13 Chlorophyll-a content (mg/m^2) on tube sections removed from each substrate station during the test period (August 1982).

DATE	STATION										TEMP. (C)
	TAYLOR BROOK					TRIBUTARY					
	Production		Uptake			Production		Uptake			
	x	x'	x	x'	P/R	x	x'	x	x'	P/R	
10/11	5.4	.90	1.2	.20	4.5	5.7	.95	1.7	.28	3.4	23
10/12	5.5	.92	-	-	-	6.4	1.10	-	-	-	
Exposure to Taylor Brook water											
10/12	3.2	.53	2.0	.33	1.6	6.1	1.02	2.1	.35	2.9	
10/13	3.2	.53	0.9	.15	3.5	7.1	1.18	1.6	.27	4.4	
10/14	1.9	.32	1.4	.23	1.4	7.1	1.18	1.9	.32	3.7	27
10/15	1.8	.30	1.4	.23	1.3	7.8	1.30	1.8	.30	4.3	28
10/16	1.2	.20	1.2	.20	1.0	6.7	1.12	1.6	.27	4.2	
10/18	1.4	.23	1.0	.17	1.4	6.5	1.08	1.6	.27	4.1	
10/19	1.0	.17	1.0	.17	1.0	6.8	1.13	1.9	.32	3.6	
10/20	1.4	.23	0.8	.13	1.8	6.6	1.10	2.5	.42	2.6	
10/21	0.9	.15	0.7	.12	1.3	6.2	1.03	4.0	.67	1.6	29
Acidified tributary											
10/21	-	-	-	-	-	7.0	1.17	3.6	.60	1.9	
10/22	0.4	.07	0.9	.15	0.4	5.6	0.93	2.8	.47	2.0	28
10/25	0.7	.12	0.6	.10	1.2	1.9	0.32	2.9	.48	0.7	27
10/26	-	-	-	-	-	0.9	0.15	2.5	.42	0.4	

Table 14 Rates of O_2 production and uptake, with corresponding P/R ratios of the algal coil communities exposed to Taylor Brook, tributary and acidified tributary waters. Recorded as mg/l (x) and converted to ug/sec (x').

DISCUSSION

Although, the various assays were instituted to investigate the feasibility of adopting the tubular artificial substrate as a routine monitoring technique, the data justify discussion. Assays will be attended individually, with the applicability of the protocol being addressed at the close of the discussion.

6.1 Fort River Assay

The Fort River assay displayed sufficient sensitivity and statistical reliability to recognize cultural eutrophication. With equal temperature and solar exposures, the slight increase in P and N concentrations (Table 1) due to the sewage outfall, accounted for a 1.8 fold enhancement in downstream substrate productivity. The sporadic outfall discharge reflected periods of peak domestic activity (dawn, noon and dusk). The application of the tubular substrate technique obviated the need for a rigorous sampling regime, since the periphyton provided a "floral memory", integrating the summation of the nutrient enrichment over time.

Variations in flow rate, (limited adjustment capabilities) caused fluctuations in O_2 evolution. Lower flow rates resulted in greater O_2 concentrations (Table 2). The diminished surface area to volume ratio (.365 m^2/l) permitted substantial dilution of evolved O_2 and consequently a much lower DO differential at faster flows. Statistical analysis revealed a highly significant regression of O_2 evolution on flow rate (Table 3). The alteration in flow rate resulted in a significantly different change in O_2 production. This was adjusted for by an analysis of covariance, which analyzed the variance in O_2 production based on a standard flow rate.

While the difference in productivity amoung the tube replicates was large, the highly significant variation between stations was much greater. Figure 6 graphically represents the substantial variation over time of both communities, demonstrating a daily rhythm, to "... intrinsic oscillations within cells coupled with environmental factors." (McCaull & Platt,1977). A distinct correlation exists between daily variations and meteorological conditions (Table 2). When each substrate value (DO differential) is plotted (Fig. 6) the individual replicates display a consistent rhythm, in spite of the statistically significant difference (Table 3). Regardless of the spatial separation, therefore the substrate replicates can be consider-

ed an extension of the same community. It is assumed then, that the statistical difference between substrate replicates could be attributed to flow rate variation and not to asynchronous communities.

6.2 Laboratory Copper Bioassay

The applicability of the tubular artificial substrate technique in the laboratory was examined through an acute bioassay with copper sulfate. Figure 7 permits interrelation of substrate replicates and treatment responses. There was no overt variation in O_2 evolution in the substrates to the range of copper treatments. Perhaps the high concentration of nutrient salts (Bolds Basic Medium,1942) competing with the copper ion for cell wall binding sites (Fitzgerald 1963) and continuous lighting (Whitton,1968) reduced copper toxicity.

In all instances, O_2 production increased to a maximum level in 8 hours. At the start of the assay, DO concentrations varied in the substrate reservoirs and could have been responsible for the variations in productivity observed between treatments (Warburg effect). The increased DO differential was attributed to greater diffusion efficiency of the evolved oxygen due to the enhanced surface area to volume ratio. In the linear tube substrates, the larger volume of water contributed a greater extent to O_2 dilution.

Substrate replicates showed a remarkably similar response to the treatments (Fig. 7). However, due to the limited sampling sequence prior to copper exposure, ANOVA could not accurately indicate the variation between coils. Future assays need not increase test water volumes but rather measurement frequency to accomodate statistical treatment of data.

6.3 Taylor Brook Field Assay

Deficiences perceived from the Fort River study were rectified in an analysis tracing the effect of mineral acidity and associated secondary consequences on the primary productivity of the lotic phytobenthos in Taylor Brook. Wet chemistry analyses suggested that substrate O_2 inhibition was attributed to pH (4.5) and aluminum (3-4 ppm) toxicity (Table 7). The extent to which both added to the elimination of O_2 production can not be ascertained from this assay. Laboratory analysis, however, conferred greater insight into this problem and will be discussed subsequently.

The actual flow rate at the periphyton-stream water interface was much less than that at the substrate axis (center line). A profile of laminar flow through a pipe depicts the flow rate gradient, decreasing from a maximum at the center line to 80% of this value at the periphyton

surface (Fig. 13).

A change in flow rate between 4-10 ml/sec (Fig. 4) yielded little effect on O_2 production, whereas flows outside this range appeared to restrict O_2 diffusion. Ambuhl (1959, 1961 & 1962) photographically demonstrated a static boundary layer between the substrate-stream water interphase. As velocity decreased, this layer increased, diminishing gaseous/nutrient diffusion and accounting for the decreased O_2 evolution at low flow rates.

Oxygen production was monitored throughout the day on 5/15/82 to determine if a diurnal rhythm existed (Fig. 14). Oxygen generation (58-84 $mg/m^2/hr$) proved comparable to values recorded in the Fort River (52-93 $mg/m^2/hr$: averaged production at each station). Low productivity rates during the morning hours was perhaps due to the lower water temperatures (10-14 C). Despite the warmer late afternoon water temperature (18 C) and maximum solar output (9000 ft-c), O_2 evolution peaked around midday, suggesting an internal regulation in productivity. It does appear, however, that the productivity peak on 5/17/82 could be attributed to abnormally high water temperature (20 C).

Light intensity was attenuated 60% within the substrates. However, at the minimum light intensity recorded during sampling (6000 ft-c), sufficient light (2400 ft-c) reached the periphyton for unrestricted rates of primary

productivity. (Phinney & McIntire (1965) cited light saturation intensities of 1500 ft-c.)

In addition to the diurnal pattern, periphyton productivity displayed a daily rhythm, alternating in high/low periods of O_2 production, similar to the Fort River assay, but with reduced amplitude (Fig. 8). Consistent meteorological conditions (light and temperature) during the midday sampling period minimized amplitudinal variations.

Examination of 5 cm tube sections served to identify the periphyton community and determine chlorophyll-a content. Such data helped to describe the community structure but the biomass gradient prohibited extrapolation to the entire substrate length. Comparisons between substrate sections, however, could be performed. The drop in chlorophyll (Fig. 9) exceeded the reduction in cell counts (Fig. 10), indicating (assuming that only live organisms remained attached to the substrate) that the loss in productivity was attributable to reduced chlorophyll (as a consequence of reduced survival) and to a lesser extent, diminished cell efficiency.

A composition comparison between test and control communities indicates that the most pronounced change was due to the persistence of Anabaena sp., Gomphonema sp. and Tabellaria sp. . Their dominance was due not to an increase

in population densities but to a drop in representation (from control levels) of Navicula sp. (¼), Achnanthes sp. (1/3), Stigeoclonium sp. (1/100) and Synedra sp. (1/10). This shift in dominance resulted after 2 weeks exposure in Taylor Brook.

The presence of a cyanophyte below a pH of 5 and the marked decrease in algal biomass is contradictory to observations of Brock (1973), Muller (1980) but consistent with those of Parsons (1968), Conroy et al (1976), Kwiatkowski & Roff (1976) and Lazarek (1981).

The reduction in substrate length (7.35 to 4.80 m) and diameter (1.25 to 0.9 cm) accounted for a 2-fold decrease in surface area (0.28 to 0.14 m^2) and volume (0.79 to 0.34 L). However, the surface area to volume ratio only increased 10% (0.37 to 0.41). As a result, the DO differentials remained comparable to the Port River values. A further reduction in diameter would be necessary to increase DO differentials.

6.4 Taylor Brook Acute Laboratory Assay

Periphyton colonized from tributary control water, exhibited slight inhibition (12%) in O_2 production after a 40 hour exposure to water collected from Taylor Brook (Table 11). This was in agreement with field data, where toxicity was only discernible after 2 days (Fig. 8).

The algal community was dominated by Anabaena sp. and Scenedesmus sp. which seemed to have demonstrated a greater productivity than the unialgal population of Ankistrodesmus falcatus. Average substrate O_2 differentials (4.3 mg/l) were 1½ times greater than those of the earlier copper assay (2.8 mg/l).

Comparison of the coefficients of variation between the copper (16.7%) and Taylor Brook (16.9%) laboratory assays, illustrates the consistently improved performance with regard to substrate replication over field measurements (29.4% Table 10). This reproducibility reflects the controlled environment provided by laboratory conditions: sustained photoperiod, constant temperature and a more manageable sampling procedure.

6.5 Taylor Brook Assay: Alternating Light/Dark Periods

The extended testing procedure instituted to measure the response of the substrate community under occluded light conditions necessitated the employment of one substrate per station. Sampling of this extent would have been impractical with replicates. A sequential sampling series permitted extended monitoring of O_2 production and respiration of two colonized substrates located in Taylor Brook and the tributary. A period of at least 20 minutes was required to achieve a reliable measure of respiration

(Table 14). This does not infer a 20 minute delay in O_2 uptake but the required interval for substrate purging of previously evolved O_2.

Productivity inhibition was similar to that of previous assays but corresponding respiration rates gave little evidence of inhibition (Fig. 11). The extent to which algal respiration contributed to community respiration could not be determined. However, unless the heterotrophic community could account for the major uptake of O_2 throughout the assay, the distinctly autotrophic P/R ratio (3-4) in the substrate community prior to transfer to Taylor Brook would indicate that tube respiration was dominated by autotrophs.

Alternating light/dark periods often resulted in elevated O_2 production upon illumination, as compared to the initial light phase output (Table 14). Reduced levels of O_2 and increased CO_2 concentrations within the envelope during the dark phase provided an optimum scenario for carbon assimilation and O_2 evolution upon exposure to light.

6.6 Taylor Brook Chronic Laboratory Assay

Genera of Scenedesmus, Anabaena, Oscillatoria and Navicula represent members of the scantly diversified substrate community. Despite the structural difference, the response of the laboratory colonized periphyton to Taylor

Brook water was comparable to field observations. When the control water was amended to a reduced pH of 4.4, it proved sufficiently toxic to cause a similar reduction in productivity, without the high aluminum concentrations observed in the test water (Fig. 12).

Upon a qualitative examination of the coil communities at the close of the experiment, it was noted that diatom and cyanophyte motility was eliminated after exposure to low pH and high aluminum concentrations of Taylor Brook. Motility remained, however, in the acidified control community. Examination of field substrates verified this observation. The excretion of assimilates has been offered as a mechanism for the gliding movement of some algae (Harper & Harper,1967). Perhaps aluminum interferes with the gliding mechanism, through impregnating the cell plasmalemma, inhibiting plasmolysis (Bohm-Tuchy,1960) and thusly excretion. Admittedly, implicating aluminum as the sole inhibitor of motility is rather tenuous in light of the numerous chemical parameters remaining to be analyzed. Nevertheless, recorded lethal aluminum levels do suggest this possibility.

Perhaps both, the increased H^+ concentration and consequent enhanced mobilization of aluminum as a result of coal leachate, were pivotal in eliminating periphyton productivity. At the ranges recorded, the aluminum species present would be dominated by the toxic Al^{+3} ion

(Smith & Hem,1972). The low pH (4.4) and high aluminum concentrations (3.8 ppm) present in Taylor Brook are well within the range cited as toxic to algae. Bringman and Kuhn (1959) determined that the aluminum toxicity threshold for Scenedesmus quadricauda is between 1.5-2.0 ppm. Hall (1980) observed structural changes in the periphyton community of Norris Brook, associated with an aluminum concentration as low as 0.4 ppm at a pH of 4.0.

The acute inhibition of O_2 production in the test community reflects perhaps a competitive inhibition of CO_2 fixation due to elevated dissolved oxygen levels (Warburg effect,1919). The control water in which the substrate community was colonized, possessed a lower DO than the test water, to which the community was exposed, on 10/13/82. Minutes after the transfer of source waters, O_2 production immediately decreased (Fig. 12). The DO differential of 2 ppm between reservoir waters, created suppressed conditions for photosynthesis (55.7-33.1 mg $O_2/m^2/hr$) while enhancing respiration (15.7-24.4 mg $O_2/m^2/hr$). True inhibition occurred 2 days after exposure, similar to previous observations.

Laboratory and field observations revealed sustained test community respiration comparable to control values. An inadvertent rise in ambient laboratory temperature, however, stimulated control community respiration without a

corresponding rise in the test community (Table 14). Further respiratory inhibition was evident upon control community exposure to acidified tributary water. A 38% decrease occurred after 5 days at a pH of 4.4. Thus, the toxicity of Taylor Brook acted upon respiration as well as photosynthesis of lotic periphyton.

CONCLUSIONS

The trial assays permitted a thorough assessment of the tubular artificial substrate technique. The research was directed towards determining the practicality of the protocol regarding its application to the monitoring of lotic water quality. The protocol served as a useful adjunct to current toxicological and limiting nutrient studies and should prove to be most attractive to water pollution control agencies for its unsophisticated methodology and economy. Routine substrate monitoring required no technical experience and the assembly consisted of easily obtainable, inexpensive materials (1$/substrate foot). Long term exposure can be implemented without continued supervision and a large monetary loss is not incurred upon damage by vandalism. The flexible nature of the lexan material made the substrates quite portable and virtually indestructible. Colonized substrates plugged with corks can be hand carried to various test sites without stress to the community. The most attractive feature depends not on determining chemical conditions from laborious representative water samples but rather incorporating extended environmental perturbations into a quantitative biological response.

The rate of substrate colonization remained consistent between assays. Observable growth occured within 1-2 weeks and was sufficiently established after 3 weeks. Laboratory glass substrates possessed similar colonization rates. But when an infusion of brain-heart was added to the culture water, the time was reduced to one week.

Peak O_2 production ranged from 120 to 160 $mg/m^2/hr$ amoung various assays. The DO differentials generated between substrates varied as did the ug/sec values. However, when presented per substrate area ($mg/m^2/hr$), productivity is similar. A comparison of the DO differentials generated in the field and laboratory, yielded a 4 fold increase of DO concentrations in smaller diameter substrates as a result of an enhanced surface area to volume ratio. Detection of stress based on changes in evolved O_2 would prove more sensitive with higher DO differentials. Results from the Taylor Brook field and laboratory assays demonstrate the consequence of reduced DO differentials. Results show that O_2 production was not entirely inhibited in the laboratory phase (Fig. 12) unlike in the field (Fig'. 8 & 11). Though in both assays productivity responded similarly to toxicity the smaller field DO differentials may have limited sensitivity. However, the Fort River substrates possessed sufficient sensitivity to transmit an increase in total phosphorous (.025-.030 ppm) and nitrate nitrogen (.08-.18 ppm)

concentrations, to a 1.8 fold increase in productivity. To obtain the maximum sensitivity possible, future field studies should incorporate substrates with diameters favoring increased DO differentials.

Oscillations in daily productivity were recorded throughout the research, establishing an amplitudinal rhythm. Sampling during the Fort River study was performed during clear and cloudy conditions. Extreme variations in productivity reflected variations in solar radiation (Table 2). The Taylor Brook assay restricted testing to days of similar solar capabilities. Although the amplitude was reduced, the oscillations remained. Laboratory communities maintained these oscillations despite constant temperatue and light intensity, suggesting a cellular mediated rhythm in primary productivity. Throughout these assays, the reproducibility between substrate replicates remained significant. Whether proven statistically or displayed graphically, the spatially separated algal communities behaved as synchronous extentions of the same community.

Round (1981) emphasized the ability to interrelate field and laboratory data. Very seldom can parameters observed under natural conditions, be studied in the labor-

atory. The laboratory version of the tubular artificial substrate technique permitted further investigation of field observations, under more manageable conditions. The option to alter physical conditions and amend test waters, provided the protocol with the unique dimension to assess effectively the biological response to stressed conditions.

Despite the usefulness of the protocol, it contains several flaws that must be addressed. It should be noted that the substrate community structure did not support extrapolation to the stream bed nor did the inferred productivity mirror the natural value. Comparison of community structure and chlorophyll-a content between substrates required extricating 5 cm sections (per sample), thusly limiting the number of samples and significance of data. The reservoir unit is recommended over the weir for providing head because fabrication of the later was time consuming and vulnerable to stream freshets.

In any constant-flow system in the laboratory, the volume of water required and the stability of dissolved CO_2 and O_2 content in the test waters throughout the assay pose great problems. Though laboratory flow rates are greatly reduced (compared to field rates), large reservoirs are required with consistent CO_2 and O_2 concentrations maintained. Discharged substrate inhabitants can not be retained and a portion of the drop in O_2 production can be related to a decrease in producers as well as stressed

behavior. Future research should quantify the discharge of tube colonizers upon exposure to stressed environments, inorder to justify productivity inhibition solely as a response and not to a reduction in numbers.

The technique gained little merit as a quantitative implement of community structure. However, its versatility in assaying cultural eutrophication, pH and heavy metal toxicity, exhibited promise as a sensitive, inexpensive tool to routinely evaluate lotic environments, in the field as well as in the laboratory.

LITERATURE CITED

Assman A.V. (1953) Role of algal periphyton in the production of organic matter in Lake Glubokoje. Trudy Vsesoyuz Gidrobiol Obch 5: 138-157.

Ambuhl H. (1959) Die bedeutung der stromungals okologischer faktor. Schweiz Z Hydrol 21: 133-264.

--------- (1961) Die stromungals physiologischer und okologischer faktor. Experimentelle untersuchunden an bachtieren. Verh int Verein Theor angew 14: 390-5.

--------- (1962) Die besonderheiten der wasserstromung in physikalischer, chemischer und biologischer hinsicht. Schweiz Z Hydrol 24: 367-382.

APHA (1980) Standard Methods for the Examination of Water and Wastewater. 14 th edition American Public Health Association New York.

Barrett G.W., Van-Dyne G.M. & Odum E.P. (1976) Stress ecology. Bio Science 26: 192-4.

Bohm-Tuchy E. (1960) Plasmalemma and the action of aluminum salts. Protoplasma 52: 108.

Bold H.C. (1942) The cultivation of algae. Bot Rev 8: 69.

Bott T.L., Brock J.T., Cushing C.E., Gregory S.V., King D. & Petersen R.C. (1978) A comparison of methods for measuring primary production and community respiration in streams. Hydrobiologia 60: 3-12.

Bringmann G. & Kuhn R. (1959) Aquatic toxicology research with protozoa as test organisms. Gesung Ing 80: 238.

Brock T. (1973) Science 179: 480.

Cairns J.Jr., Lanza G.R. & Parker B.C. (1973) Pollution related structural and functional changes in aquatic communities with emphasis on freshwater algae and protozoa. Proc Acad Nat Sci Phila 124(4-6):79-127

Cummins K.W. (1974) Structure and function of stream ecosystems. Bio Sci 24: 631-641.

Conroy H., Hawley K., Keller W. & Lafrance C. (1976) Influences of the atmosphere on lakes in the Sudbury area. Journal of Great Lakes Research 2 (supplement 1) 146-165.

Dunnett C.W. (1955) A multiple comparisons procedure for comparing several treatments with a control. J Amer Statist Ass 50: 1096-1121.

Elwood J.W. & Nelson D.J. (1972) Periphyton production and grazing rates in a stream measured with ^{32}P material balance method. Oikos 23: 295-303.

Fischer S.G. (1977) Organic matter processing by a stream-segment ecosystem: Fort River, Massachusetts, USA. Int Revue ges Hydrobiol 62: 701-727.

Fitzgerald G.P. & Faust S.L. (1963) Factors affecting the algicidal and algistatic properties of copper. Appl Microbiol 11(4): 345-351.

Gerhardt D.Z., Anderson S.M. & Richter J. (1977) Toxicity bioassays with periphyton communities: design of experimental streams. Water Res 11: 567-570.

Hall R.J., Likens G.E., Fiance S.B. & Hendrey G.R. (1980) Experimental acidification of a stream in the Hubbard Brook Experimental Forest, New Hampshire. Ecology 61: 976-989.

Harper M.A. & Harper J.F. (1967) Measurements of diatom adhesion and their relationship with movement. Br Phycol Bull 3: 195-207.

Hentschel E. (1916) Hafen Mitt Zool Mus Hamburg 33: 1-172.

Holm-Hansen O. (1973) In Estuarine and Microbial Ecology edited by Stevenson H.L. & Colwell R.R. pg. 73-78 University of South Carolina Press Columbia SC.

Kaplan E. (1981) Rock, soil and water quality. Relationships and implications for effects of acid precipitation on surface water in the Northeastern United States. Env Sci and Tech 15:539.

Kelly M.G., Hornberger G.M. & Cosby B.J. (1974) Continuous automated measurement of rates of photosynthesis and respiration in an undisturbed river community. Limnol Oceanogr 19: 305-312.

Kolkwitz R. & Marsson M. (1908) Ecology of plant saprobia. Rep German Bot Soc 26: 505-509.

Kwiatkowski R.E. & Roff J.C. (1976) Can J Bot 54: 2546.

Lampkin A.J. III (1982) Algal distribution in a small intermitant stream receiving acid mine-drainage. J Phycol 18: 196-199.

Lazarek S. (1982) Structure and productivity of epiphytic algal communities on Lobelia Dortmanns L. in acidified and limed lakes. Water Air and Soil Pollution 18: 333-342.

Marker A.F.H. (1976) The benthic algae of some streams in Southern England. J of Ecol 64: 359-373.

McCaull W.A. & Platt T. (1977) Diel variations in the photosynthetic parameters of coastal marine phytoplankton. Limnol Oceanogr 22: 723-31.

McIntire C.D., Garrison R.L., Phinney H.K. & Warren C.E. (1964) Primary production in laboratory streams. Limnol Oceanogr 9:92-102.

McIntire C.D. & Phinney H.K. (1965) Laboratory studies of periphyton productivity and community metabolism in lotic environments. Ecol Monogr 35: 237-258.

McIntire C.D. (1975) In River Ecology edited by Whitton B.A. pg 403-430.

McConnell W.J. & Sigler W.F. (1959) Chlorophyll and productivity in a mountain river. Limnol Oceanogr 4:335.

Muller P. (1980) Effects of artificial acidification on the growth of periphyton. Can J of Fish and Aquatic Sci 37: 355-363.

Odum H.T. (1956) Primary production in flowing waters. Limnol Oceanogr 2: 102-117.

--------- (1957) Primary productivity measurements in eleven Florida springs and a marine turtle grass community. Limnol Oceanogr 2: 85-97.

--------- (1964) The new ecology. Bio Sci 14: 14-16.

--------- (1977) The emergence of ecology as a new integrative discipline. Science 195: 1289-1293.

Parsons J.D. (1968) The effects of acid strip-mine effluents on the ecology of a stream. Arch Hydrobiol 65: 25-50.

Patrick R., Hohn M.H. & Wallace J.H. (1954) A new method for determining the pattern of the diatom flora. Notul Nat 259: 1-12.

Pennak R.W. & Lavelle J.W. (1979) Net primary productivity in a Colorado Mt. stream. Hydrobiologia 66: 227-335.

Pfeifer R.F. (1975) Some factors affecting primary productivity of stream riffle communities. Arch Hydrobiol 75: 306-317.

Phinney H.K. & McIntire C.D. (1965) Effect of temperature on the metabolism of periphyton communities developed in laboratory streams. Limnol Oceanogr 10: 341-344.

Round F.E. (1981) The Ecology of Algae Cambridge University Press Great Britain.

Sladeckova A. (1962) Limnological investigation methods for the periphyton (Aufwuchs) community. Bot Rev 286-350.

Smith R.W. & Hem J.D. (1972) Effect of aging on aluminum hydroxide complexes in dilute aqueous solutions. U.S. Geol Surv Water Supply Pap no. 1827

Taylor Brook Survey (1982) River Pollution- Biological Survey Methods, Fall 1981. Environmental Science 510 University of Massachusetts, Amherst, Mass.

Thomas N.A. & O'Connell R.L. (1966) A method for measuring primary productivity by stream benthos. Limnol Oceanogr 11: 386-392.

Tippett R. (1970) Artificial surfaces as a method of studying populations of benthic micro-algae in fresh water Br Phycol J 5: 187-199.

U.S. EPA (1971) Algal Assay Procedure: Bottle Test National Eutrophication Research Program Corvallis OR.

Vollenweider R.A. (1969) A manual on methods for measuring primary production in aquatic environments. 213 pp. IBP handbook no. 12. Blackwell, Oxford.

Warburg O. (1919) Uber die Geschwindigeit der photochemischen kohlensaurezersetzung in lebenden Zellen. Biochem Z 100:230.

Waters T.F. (1961) Notes on the chlorophyll method of estimating the photosynthetic capacity of stream periphyton. Limnol Oceanogr 6: 486-488.

Weitzel R.L. (Ed) (1979) Methods and measurements of periphyton communities: A review. ASTM STP 690 American Society for Testing and Materials.

Whitton B.A. (1968) Effect of light intensity on toxicity of various substances to Anacystis nidulans. Plant and Cell Physiol 9: 23-26.

---------- (1975) River Ecology University of Calif Press Berkely and Los Angeles.